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Lagudah et al.

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(54) **RESISTANCE GENES**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 129 days.

This patent is subject to a terminal disclaimer.

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(22) Filed: **Oct. 9, 2013**

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Related U.S. Application Data

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(51) **Int. Cl.**

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C12N 15/29	(2006.01)
C07K 14/415	(2006.01)
A01H 5/10	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 15/8282*** (2013.01); ***A01H 5/10*** (2013.01); ***C07K 14/415*** (2013.01); ***C12N 5/14*** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to polynucleotides encoding adult plant pathogen resistance proteins. Also provided are transgenic plants expressing these polynucleotides to enhance the resistance of the plants to pathogens.

20 Claims, 9 Drawing Sheets

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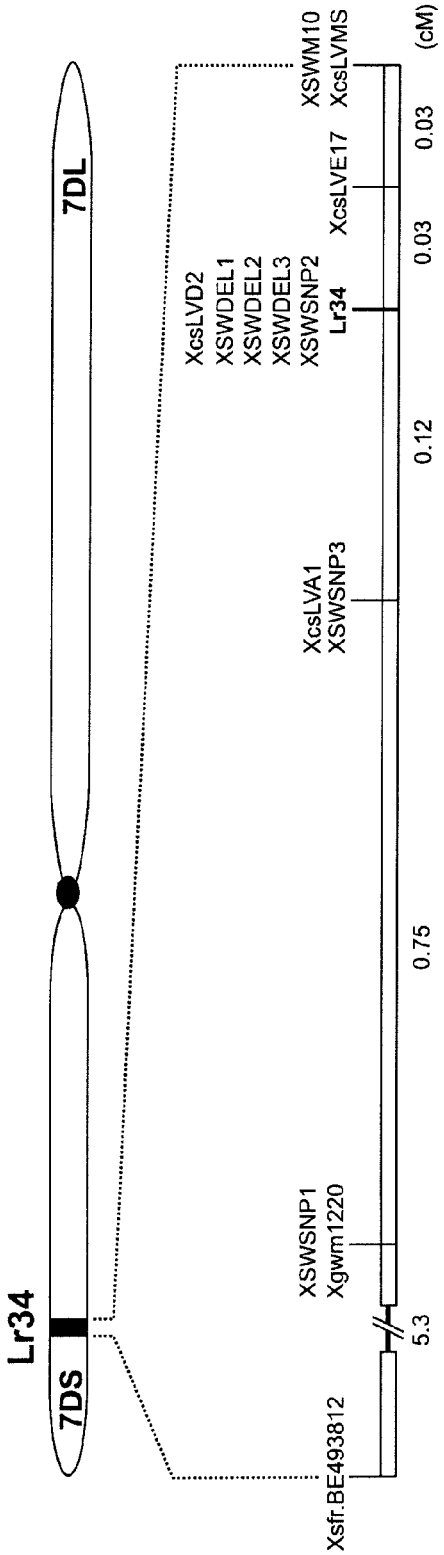
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Figure 1

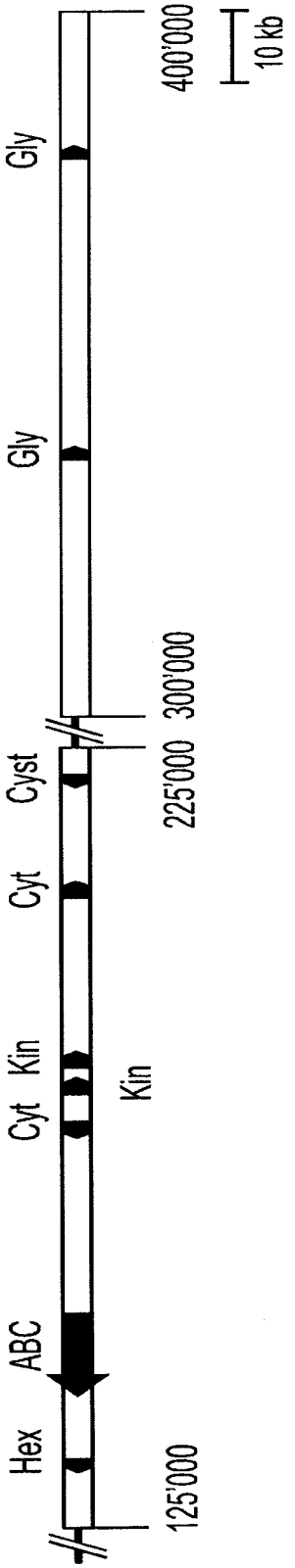
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Figure 2

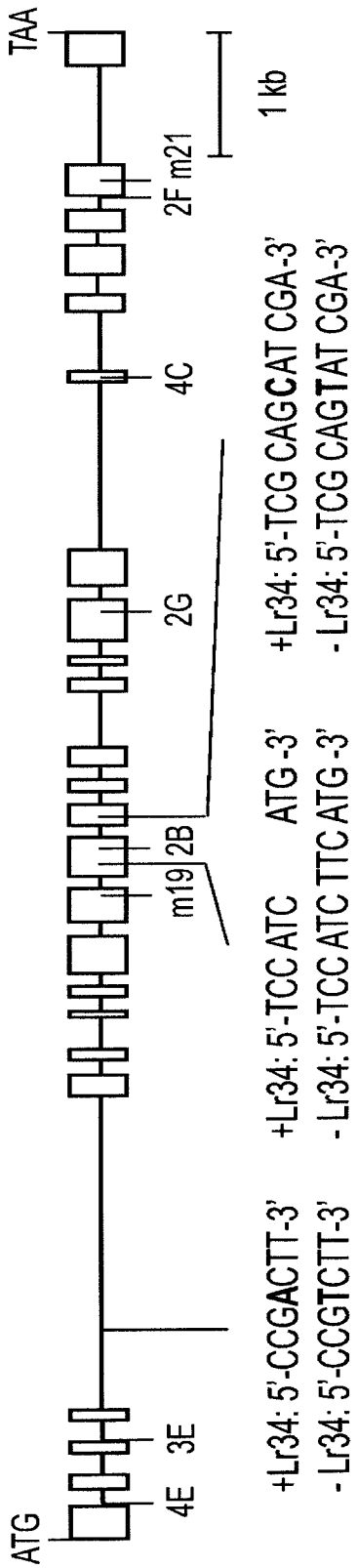
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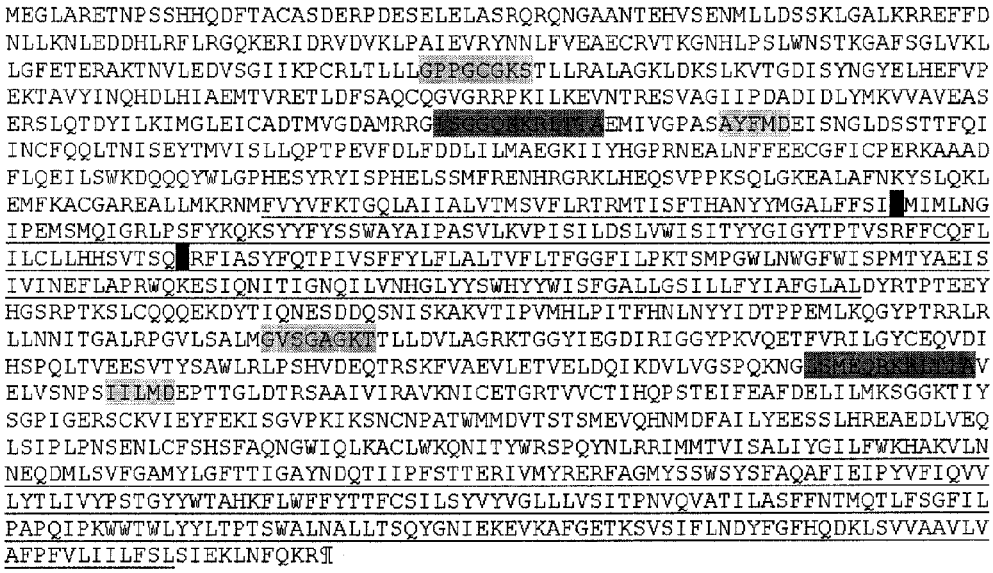
Figure 3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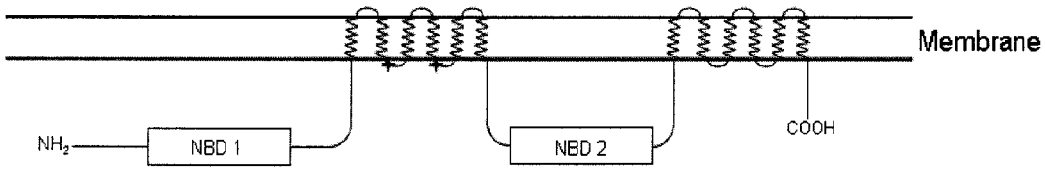

Figure 5

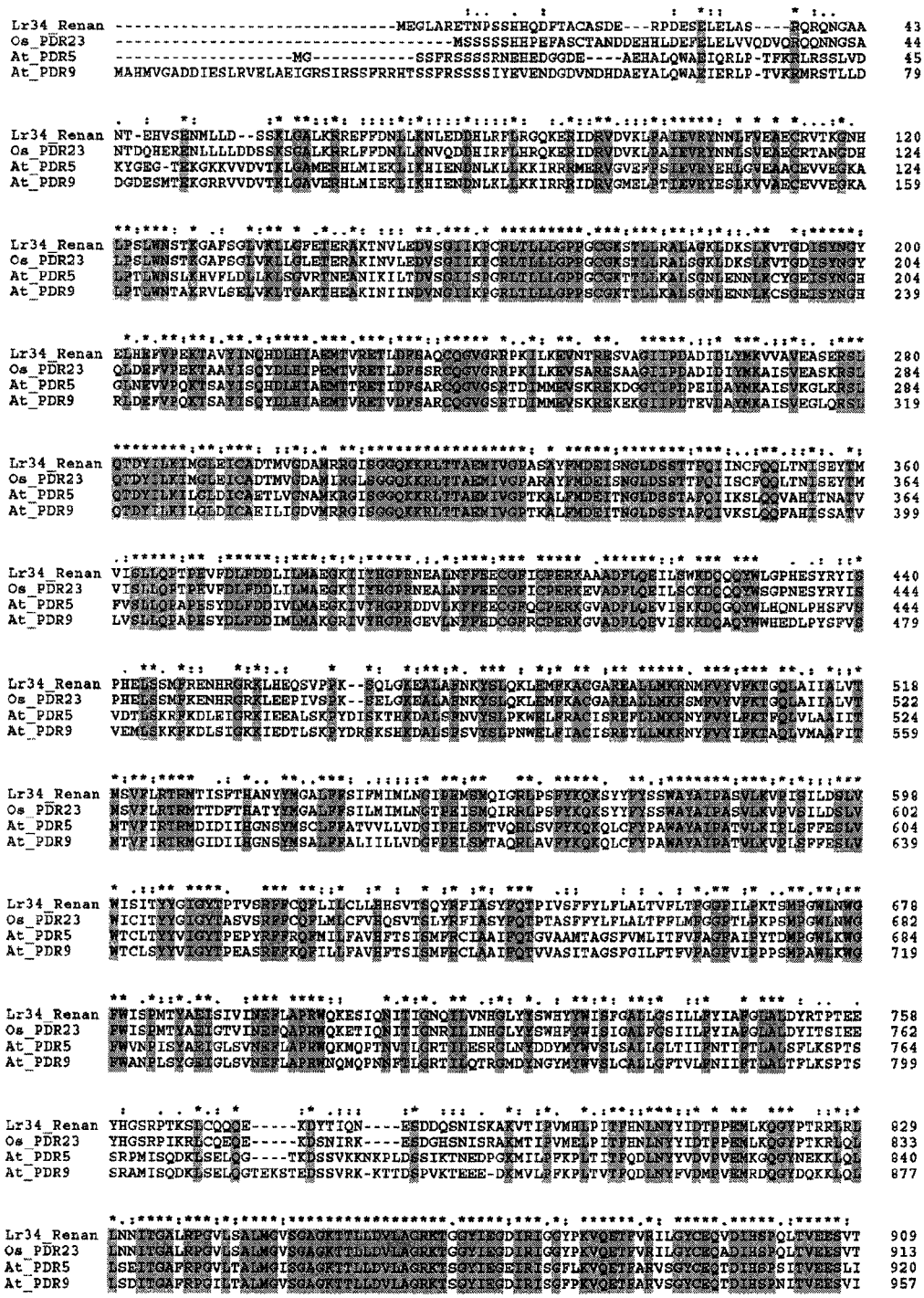

Figure 6

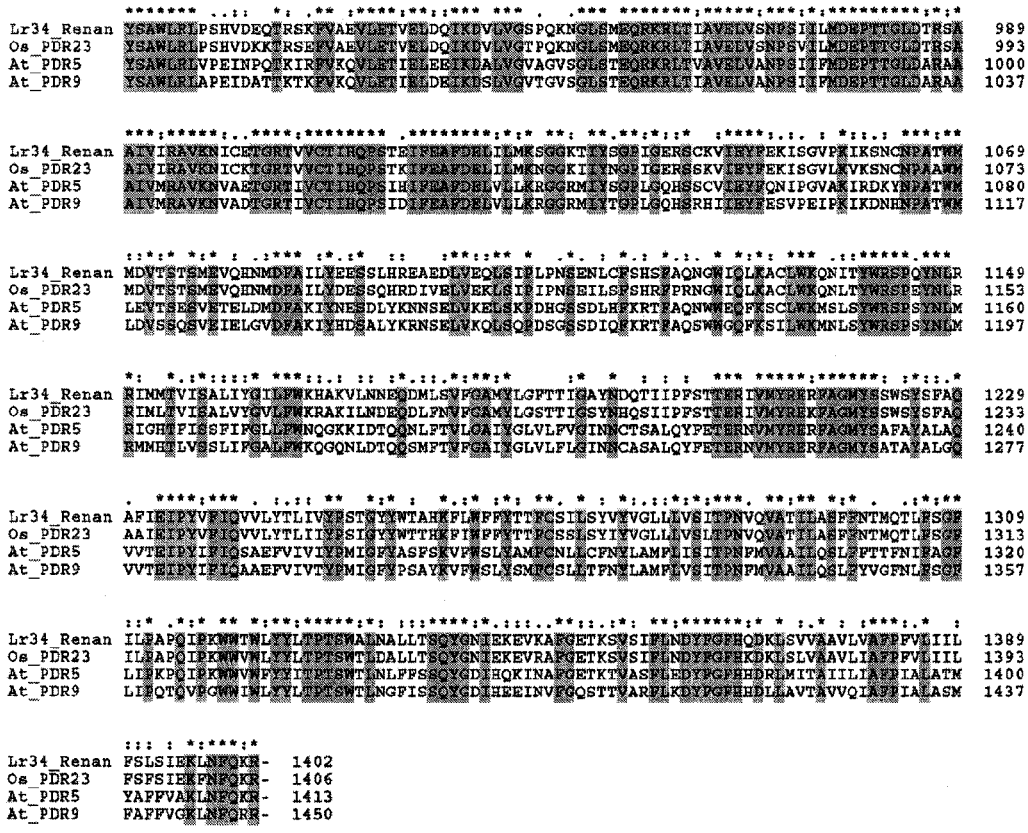

Figure 6 (continued)

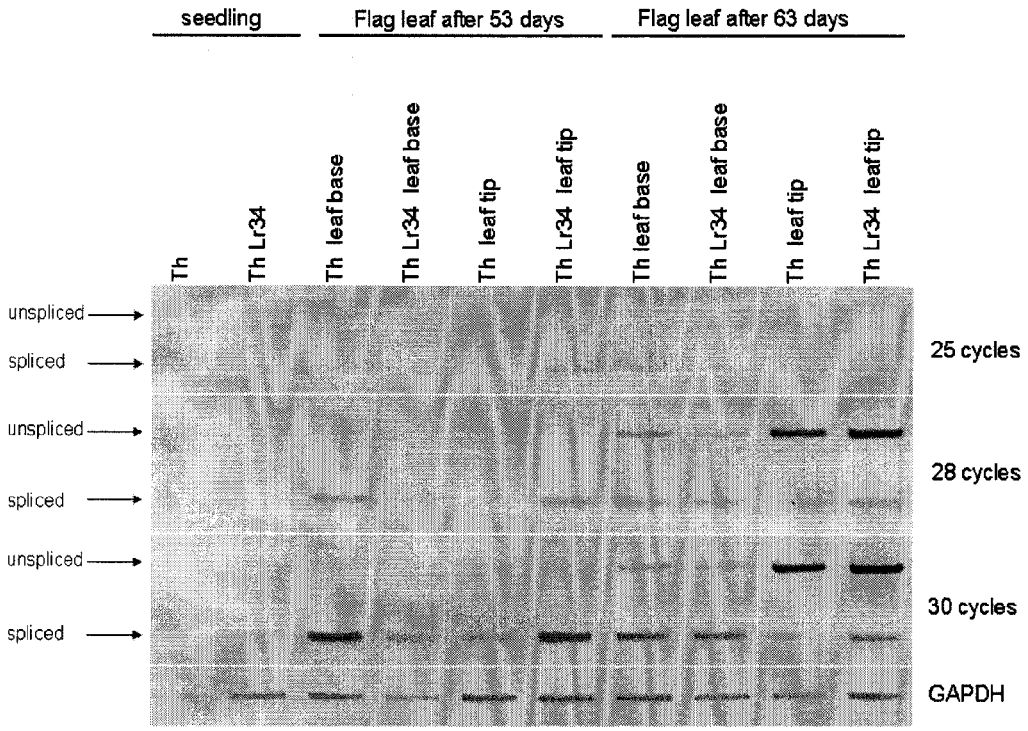
seedling
Flag leaf after 53 days
Flag leaf after 63 days
Th
Th Lr34
Th leaf base
Th Lr34 leaf base
Th leaf tip
Th Lr34 leaf tip
Th leaf base
Th Lr34 leaf base
Th leaf tip
Th Lr34 leaf tip
unspliced
spliced
unspliced
spliced
unspliced
spliced
25 cycles
28 cycles
30 cycles
GAPDH

Figure 7

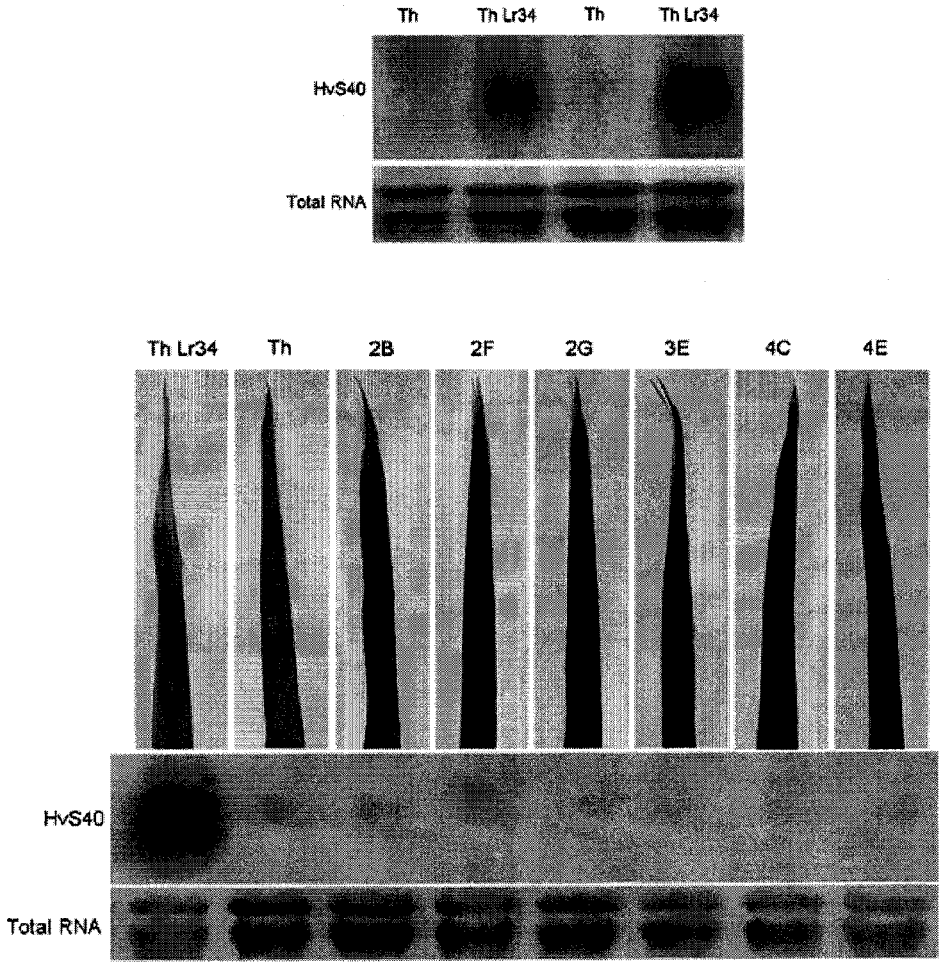
Th
Th Lr34
Th
Th Lr34
HvS40
Total RNA
Th Lr34
Th
2B
2F
2G
3E
4C
4E
HvS40
Total RNA

Figure 8

RESISTANCE GENES

FIELD OF THE INVENTION

The present invention relates to polynucleotides encoding adult plant pathogen resistance proteins. Also provided are transgenic plants expressing these polynucleotides to enhance the resistance of the plants to pathogens.

BACKGROUND OF THE INVENTION

Numerous genes conferring resistance to pathogens have been identified and used in plant breeding. However, single-gene pathogen resistance in plants often becomes ineffective due to the emergence of new virulent races of the disease agent. In contrast, durable disease resistance in plants is generally thought to be controlled by multiple genes.

The wheat (*Triticum aestivum*) quantitative trait locus, Lr34, provides durable adult plant resistance to the biotrophic fungi causing the diseases leaf rust, stripe rust, stem rust and powdery mildew (Dyck, 1977 and 1987; German and Kolmer, 1992; Bossolini et al. 2006; Spielmeyer et al. 2008). This is despite the limitation that it is not effective at the seedling stage under normal field conditions. Cultivars with the resistance locus Lr34 such as Frontana have had effective durable resistance to the leaf rust fungus *Puccinia triticina* Eriks (Dyck et al., 1966; Singh and Rajaram, 1994). To date, isolates of *P. triticina* with complete virulence to Lr34 have not been detected (Kolmer et al., 2003).

Lr34 resistance has remained genetically inseparable from Yr18 that confers resistance to stripe rust (*P. striiformis*) (Singh, 1992a; McIntosh, 1992). Co-segregation of Lr34/Yr18 with other traits such as leaf tip necrosis (Ltn1), powdery mildew (recently designated Pm38), tolerance to barley yellow dwarf virus (Bdvl) and spot blotch (*Bipolaris sorokiniana*) have been documented (Singh, 1992a, b; McIntosh, 1992; Joshi et al., 2004; Spielmeyer et al., 2005; Liang et al., 2006). These multi-pathogen resistance traits have made the Lr34/Yr18 locus one of the most valuable gene regions for disease resistance breeding in wheat.

A few rust resistance genes have been isolated and cloned from wheat (Feuillet et al., 2003; Huang et al., 2003; Cloutier et al., 2007) and other cereals (Collins et al., 1999; Brueggeman et al., 2002) and are predominantly from the nucleotide binding site-leucine rich repeat (NB-LRR) class of major resistance (R) genes. The only known exception is the barley Rpg1 rust resistance gene which encodes a protein kinase. These genes encode gene-for-gene resistance against single pathogens and generally lead to hypersensitive responses in the plant tissues upon infection. In contrast, Lr34 confers a broad spectrum resistance against several obligate biotrophic pathogens including fungi from the Ascomycetes and Basidiomycetes. Rubiales and Niks (1995) reported that Lr34 is associated with reduced intercellular hyphal growth but not with a hypersensitive response or papilla formation.

The molecular basis of quantitative non-race-specific, adult plant pathogen resistance-type or partial resistance encoded by genetic systems such as, for example, Lr34 therefore remains unknown.

SUMMARY OF THE INVENTION

The present inventors have identified genes and polypeptides which confer enhanced plant pathogen resistance to adult plants.

Accordingly, the present invention provides a transgenic plant which has integrated into its genome an exogenous polynucleotide encoding an adult plant pathogen resistance polypeptide and/or an exogenous polynucleotide which increases transcription of an endogenous gene encoding an adult plant pathogen resistance polypeptide.

In a preferred embodiment, the plant has accelerated senescence of flag leaf tips when compared to an isogenic plant lacking the exogenous polynucleotide.

In another preferred embodiment, the plant has enhanced resistance to a plant pathogen when compared to an isogenic plant lacking the exogenous polynucleotide.

In yet a further preferred embodiment, the polypeptide comprises amino acids having a sequence as provided in SEQ ID NO:1, a biologically active fragment thereof, or an amino acid sequence which is at least 40% identical, more preferably at least 80% identical, more preferably at least 90% identical, and even more preferably at least 95% identical, to SEQ ID NO:1. More preferably, the polypeptide comprises amino acids having a sequence as provided in SEQ ID NO:1.

In another preferred embodiment, the polynucleotide comprises nucleotides having a sequence as provided in SEQ ID NO:2, a sequence which is at least 40% identical to SEQ ID NO:2, and/or a sequence which hybridizes to SEQ ID NO:2.

In another embodiment, the exogenous polynucleotide which increases transcription of an endogenous gene encoding an adult plant pathogen resistance polypeptide is a genetic element, such as a promoter, which enhances the function of the endogenous gene promoter. Alternatively, the exogenous polynucleotide which increases transcription of an endogenous gene encoding an adult plant pathogen resistance polypeptide encodes a transcription factor which enhances expression of the endogenous gene.

Preferably, the plant is a cereal plant. Examples of transgenic cereal plants of the invention include, but are not limited to wheat, barley, maize, rice, oats and triticale. In a particularly preferred embodiment, the plant is wheat.

Examples of plant pathogens include, but are not limited to viruses, bacteria and fungi.

In a preferred embodiment, the pathogen is a biotrophic fungus. Examples of biotrophic fungi include, but are not limited to, *Fusarium graminearum* (which causes head blight), *Erysiphe graminis f.* sp. *tritici* (which causes powdery mildew), *Bipolaris sorokiniana* (which causes spot blotch), *Puccinia graminis f.* sp. *tritici* (which causes stem rust), *Puccinia striiformis* (which causes stripe rust) and *Puccinia recondite f.* sp. *tritici* (which causes leaf rust).

In an embodiment, the pathogen is barley yellow dwarf virus (BYDV).

In an embodiment, the plant comprises one or more further exogenous polynucleotides encoding a plant pathogen resistance polypeptide. Examples of such genes include, but are not limited to, Lr1, Lr3, Lr2a, Lr3ka, Lr11, Lr13, Lr16, Lr17, Lr18, Lr21 and LrB.

In another aspect, the present invention provides a process for identifying a polynucleotide encoding a plant pathogen resistance polypeptide comprising:

(i) obtaining a polynucleotide operably linked to a promoter, the polynucleotide encoding a polypeptide comprising amino acids having a sequence as provided in SEQ ID NO:1, a biologically active fragment thereof, or an amino acid sequence which is at least 40% identical to SEQ ID NO:1,

(ii) introducing the polynucleotide into a plant,

(iii) determining whether the level of resistance to a plant pathogen is modified relative to an isogenic plant lacking the polynucleotide, and

(iv) optionally, selecting a polynucleotide which when expressed enhances resistance to the plant pathogen.

Preferably, the polynucleotide comprises nucleotides having a sequence as provided in SEQ ID NO:2, a sequence which is at least 40% identical to SEQ ID NO:2, and/or a sequence which hybridizes to SEQ ID NO:2.

Preferably, the plant is a cereal plant.

Preferably, the cereal plant is a wheat plant.

In a preferred embodiment, the polypeptide is a plant polypeptide or mutant thereof.

In a further embodiment, step (ii) further comprises stably integrating the polynucleotide operably linked to a promoter into the genome of the plant.

In yet another aspect, the present invention provides a substantially purified and/or recombinant adult plant pathogen resistance polypeptide.

In a preferred embodiment, the polypeptides comprises amino acids having a sequence as provided in SEQ ID NO:1, a biologically active fragment thereof, or an amino acid sequence which is at least 40% identical, more preferably at least 80% identical, more preferably at least 90% identical, and even more preferably at least 95% identical, to SEQ ID NO:1.

In a preferred embodiment, the polypeptide lacks a phenylalanine residue or any amino acid at a position corresponding to amino acid number 546 of SEQ ID NO:4.

In another preferred embodiment, the polypeptide has an amino acid other than a tyrosine residue at a position corresponding to amino acid number 634 of SEQ ID NO:4. More preferably, the polypeptide comprises a histidine residue at a position corresponding to amino acid number 634 of SEQ ID NO:4.

Also provided is a fusion protein further comprising at least one other polypeptide sequence. The at least one other polypeptide may be, for example, a polypeptide that enhances the stability of a polypeptide of the present invention, or a polypeptide that assists in the purification or detection of the fusion protein.

In a further aspect, the present invention provides an isolated and/or exogenous polynucleotide comprising nucleotides having a sequence as provided in SEQ ID NO:2, a sequence which is at least 40% identical to SEQ ID NO:2, a sequence encoding a polypeptide of the invention, and/or a sequence which hybridizes to SEQ ID NO:2.

Preferably, the polynucleotide comprises a sequence of nucleotides which hybridizes to SEQ ID NO:2 under stringent conditions.

Preferably, the polynucleotide hybridizes along the full length of a polynucleotide consisting of nucleotides having the sequence of SEQ ID NO:2.

Preferably, the polynucleotide encodes an adult plant pathogen resistance polypeptide.

In a further aspect, the present invention provides a chimeric vector comprising the polynucleotide of the invention.

Preferably, the polynucleotide is operably linked to a promoter.

In a further aspect, the present invention provides a recombinant cell comprising an exogenous polynucleotide of the invention and/or a vector of the invention.

The cell can be any cell type such as, but not limited to, a plant cell, a bacterial cell, an animal cell or a yeast cell.

Preferably, the cell is a plant cell. More preferably, the plant cell is a cereal plant cell. Even more preferably, the cereal plant cell is a wheat cell.

In a further aspect, the present invention provides a method of producing the polypeptide of the invention, the method comprising expressing in a cell or cell free expression system the polynucleotide of the invention.

Preferably, the method further comprises isolating the polypeptide.

In yet another aspect, the present invention provides a transgenic non-human organism comprising an exogenous polynucleotide of the invention, a vector of the invention and/or a recombinant cell of the invention.

Preferably, the transgenic non-human organism is a plant.

In another aspect, the present invention provides a method of producing the cell of the invention, the method comprising the step of introducing the polynucleotide of the invention, or a vector of the invention, into a cell.

Preferably, the cell is a plant cell.

In a further aspect, the present invention provides a method of producing a transgenic plant, the method comprising regenerating a transgenic plant from the cell of the invention.

Also provided is the use of the polynucleotide of the invention, or a vector of the invention, to produce a recombinant cell.

Further, provided is the use of the polynucleotide of the invention, or a vector of the invention, to produce a transgenic plant.

Preferably, the transgenic plant has accelerated senescence of flag leaf tips when compared to an isogenic plant lacking the exogenous polynucleotide and/or vector, and/or has enhanced resistance to a plant pathogen when compared to an isogenic plant lacking the exogenous polynucleotide and/or vector.

In another aspect, the present invention provides a transgenic plant, or progeny thereof, produced using a method of the invention.

In a further aspect, the present invention provides a plant part of the plant of the invention.

Examples of such plant parts include, but are not limited to, leaves, roots, stems and/or seeds. In a preferred embodiment, the plant part is a seed that comprises an exogenous polynucleotide encoding an adult plant pathogen resistance polypeptide.

In another aspect, the present invention provides a method of producing a plant part, the method comprising,

a) growing a plant of the invention, and

b) harvesting the plant part.

In yet a further aspect, the present invention provides a method of producing flour, wholemeal, starch or other product obtained from seed, the method comprising;

a) obtaining seed of the invention, and

b) extracting the flour, wholemeal, starch or other product.

In another aspect, the present invention provides a product produced from a plant of the invention and/or a plant part of the invention.

In one embodiment, the product is a food product. Examples include, but are not limited to, flour, starch, leavened or unleavened breads, pasta, noodles, animal fodder, breakfast cereals, snack foods, cakes, malt, beer, pastries and foods containing flour-based sauces.

In another embodiment, the product is a non-food product. Examples include, but are not limited to, films, coatings, adhesives, building materials and packaging materials.

In a further aspect, the present invention provides a method of preparing a food product of the invention, the method comprising mixing seed, or flour, wholemeal or starch from said seed, with another ingredient.

In a further aspect, the present invention provides a method of preparing malt, comprising the step of germinating the seed of the invention.

In another embodiment, the present invention provides a composition comprising a polypeptide of the invention, a

polynucleotide of the invention, a vector of the invention, and/or recombinant cell of the invention, and one or more acceptable carriers.

In another aspect, the present invention provides a substantially purified antibody, or fragment thereof, that specifically binds a polypeptide of the invention.

Also provided is a method of identifying a compound that binds to a polypeptide comprising amino acids having a sequence as provided in SEQ ID NO:1 or SEQ ID NO:4, a biologically active fragment thereof, or an amino acid sequence which is at least 40% identical to SEQ ID NO:1 and/or SEQ ID NO:4, the method comprising:

i) contacting the polypeptide with a candidate compound, and

ii) determining whether the compound binds the polypeptide.

Further, provided is a method of identifying a compound that is transported across a cell membrane by a polypeptide comprising amino acids having a sequence as provided in SEQ ID NO:1 or SEQ ID NO:4, a biologically active fragment thereof, or an amino acid sequence which is at least 40% identical to SEQ ID NO:1 and/or SEQ ID NO:4, the method comprising:

i) contacting the polypeptide present in a cell membrane with a candidate compound,

ii) determining whether the polypeptide transports the compound across the cell membrane.

Preferably, the polypeptide is expressed in a cell.

Preferably, the cell is a plant cell.

In an embodiment, the method further comprises comparing the binding, and/or transport, of the compound to a first polypeptide comprising an amino acid sequence provided as SEQ ID NO:1 to a second polypeptide comprising an amino acid sequence provided as SEQ ID NO:4.

In a further aspect, the present invention provides an isolated and/or exogenous polynucleotide which, when present in a cell of a plant, decreases the expression of at least one gene that hybridises under stringent conditions to a nucleic acid molecule encoding a polypeptide comprising amino acids having a sequence as provided in SEQ ID NO:1 or SEQ ID NO:4, a biologically active fragment thereof, or an amino acid sequence which is at least 40% identical to SEQ ID NO:1 and/or SEQ ID NO:4, said decreased expression being relative to an otherwise isogenic cell of a plant that lacks said polynucleotide.

In an embodiment, the polynucleotide encodes an adult plant pathogen resistance polypeptide.

Preferably, the polynucleotide of this aspect is operably linked to a promoter capable of directing expression of the polynucleotide in a cell of a plant.

Preferably, the polynucleotide of this aspect is an antisense polynucleotide, a sense polynucleotide, a catalytic polynucleotide, an artificial microRNA or a duplex RNA molecule.

In a further aspect, the present invention provides a method of identifying a plant comprising a gene encoding an adult plant pathogen resistance polypeptide, the method comprising

i) amplifying and/or sequencing, from a sample of the plant, at least a portion of a polynucleotide which encodes a polypeptide comprising amino acids having a sequence as provided in SEQ ID NO:1 or SEQ ID NO:4, a biologically active fragment thereof, or an amino acid sequence which is at least 40% identical to SEQ ID NO:1 and/or SEQ ID NO:4,

ii) determining if the plant comprises a polynucleotide encoding an adult plant pathogen resistance polypeptide.

As will be apparent, preferred features and characteristics of one aspect of the invention are applicable to many other aspects of the invention.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

The invention is hereinafter described by way of the following non-limiting Examples and with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

FIG. **1**. Consensus genetic map of wheat chromosome 7D including Lr34 based on three high-resolution mapping populations defined a 0.15 cM target interval for Lr34 between XSWSNP3 and XcsLVE17. Relative positions of molecule markers are shown together with the observed recombinational distances in cM.

FIG. **2**. Schematic of expanded view of part of wheat chromosome 7DS between XSWSNP3 and XcsLVE17 showing relative positions of open reading frames. The corresponding physical target interval sequenced on the +Lr34 cultivar 'Chinese Spring' contained ten candidate genes, nine of which are represented in the Figure by arrows. Numbers refer to the respective nucleotide positions within the 420 kb sequenced interval. Abbreviations: Gly, glycosyl transferase; Cyst, cysteine proteinase; Cyt, Cytochrome P450; LecK, lectin kinase; ABC, ABC transporter; Hex, hexose carrier.

FIG. **3**. Gene structure of Lr34. Open boxes indicate exons, while introns are shown as adjoining lines. Marks indicate the positions of mutation sites of the mutants labelled 2B, 2F, 2G, 3E, 4C, 4D, m19 and m21. The three sequence differences between susceptible and resistant alleles of Lr34 are indicated: +Lr34 resistant allele from Chinese Spring, –Lr34 susceptible allele from Renan.

FIG. **4**. Lr34 protein sequence and polymorphisms between resistant and susceptible cultivars. Amino acid sequence of the Lr34 protein (susceptible allele) from cultivar 'Renan'. The two amino acids that are altered in the resistant allele are highlighted. Other boxes indicate the positions of the highly conserved motifs within the nucleotide binding domains. Motifs: "Walker A" GPPGCGKS (amino acids 168-175) (SEQ ID NO:50) and GVSGAGKT (amino acids 847-854) (SEQ ID NO:51); "ABC signature" ISGGQKKRLTTA (amino acids 307-318) (SEQ ID NO:52) and LSMEQRKRLTIA (amino acids 954-965) (SEQ ID NO:53); "Walker B" AYFMD (amino acids 327-331) (SEQ ID NO:54) and IILMD (amino acids 974-978) (SEQ ID NO:55). Amino acid changes in the resistant allele of Lr34 in wheat cultivar Chinese Spring are deletion of amino acid 546 (Phe (F)) and substitution of amino acid 634 (tyrosine (Y)) to histidine. Underlined portions are the two transmembrane domains (amino acids 502-750 and 1152-1392).

FIG. **5**. Schematic representation of the Lr34 protein showing the two nucleotide binding domains (NBD) and the two transmembrane domains. The two diagnostic polymorphisms between resistant and susceptible alleles in the first transmembrane domain are indicated by stars.

FIG. **6**. Lr34 amino acid sequence alignment. Alignment of Lr34 of cultivar Renan with rice PDR23 (Os12g0512700) (SEQ ID NO:47) and *Arabidopsis* PDR5 (At3g53480) (SEQ ID NO:48) and PDR9 (At2g37280) (SEQ ID NO:49). Resi-

dues identical in all the four transporters are indicated. Rice PDR23 has been newly annotated according to the wheat Lr34 cDNA.

FIG. **7**. Expression analysis of Lr34. Semi-quantitative RT-PCR using a probe from the 5' end of the gene. Leaves of the near isogenic lines 'Thatcher' and 'Thatcher Lr34' were harvested at the seedling stage after 14 days and of adult flag leaves on 53 and 63 days old plants. Adult leaves were halved to separately study expression levels of leaf base and leaf tip. Abbreviations: TH='Thatcher'; TH Lr34='Thatcher Lr34'; GAPDH=Glyceraldehyde 3-phosphate dehydrogenase.

FIG. **8**. Lr34 regulates senescence of flag leaves. Northern Blot using HvS40 on 63 days old flag leaves of the near isogenic lines 'Thatcher' and 'Thatcher Lr34' and the azide induced Lr34 mutants 2B, 2F, 2G, 3E, 4C and 4E. TH='Thatcher'; TH Lr34=' Thatcher Lr34'.

KEY TO THE SEQUENCE LISTING

SEQ ID NO:1—Amino acid sequence of Lr34 protein (resistant allele) from *Triticum aestivum* cv Chinese spring.
SEQ ID NO:2—Nucleotide coding sequence for Lr34 from *Triticum aestivum* cv Chinese spring.
SEQ ID NO:3—Nucleotide sequence of the Lr34 gene (genomic sequence) from *Triticum aestivum* cv Chinese spring. 24 exons are present which encode the Lr34 protein:
exon 1 starts at nucleotide 3042 and ends at nucleotide 3316;
exon 2 starts at nucleotide 3416 and ends at nucleotide 3539;
exon 3 starts at nucleotide 3693 and ends at nucleotide 3778;
exon 4 starts at nucleotide 3934 and ends at nucleotide 4018;
exon 5 starts at nucleotide 6527 and ends at nucleotide 6686;
exon 6 starts at nucleotide 6784 and ends at nucleotide 6860;
exon 7 starts at nucleotide 7119 and ends at nucleotide 7172;
exon 8 starts at nucleotide 7271 and ends at nucleotide 7361;
exon 9 starts at nucleotide 7439 and ends at nucleotide 7740;
exon 10 starts at nucleotide 7833 and ends at nucleotide 8108;
exon 11 starts at nucleotide 8187 and ends at nucleotide 8497;
exon 12 starts at nucleotide 8583 and ends at nucleotide 8743;
exon 13 starts at nucleotide 8825 and ends at nucleotide 8928;
exon 14 starts at nucleotide 9015 and ends at nucleotide 9168;
exon 15 starts at nucleotide 9606 and ends at nucleotide 9513;
exon 16 starts at nucleotide 9808 and ends at nucleotide 9581;
exon 17 starts at nucleotide 9985 and ends at nucleotide 10317;
exon 18 starts at nucleotide 10427 and ends at nucleotide 10717;
exon 19 starts at nucleotide 12159 and ends at nucleotide 12242;
exon 20 starts at nucleotide 12711 and ends at nucleotide 12844;
exon 21 starts at nucleotide 12995 and ends at nucleotide 13222;
exon 22 starts at nucleotide 13318 and ends at nucleotide 13489;
exon 23 starts at nucleotide 13569 and ends at nucleotide 13823; and
exon 24 starts at nucleotide 14613 and ends at nucleotide 14939.
SEQ ID NO:4—Amino acid sequence of Lr34 protein (susceptible allele) from *Triticum aestivum* "Renan".
SEQ ID NO:5—Nucleotide coding sequence for Lr34 (susceptible allele) from *Triticum aestivum* "Renan".
SEQ ID NO:6—Genomic DNA for *Aegilops tauschii* Lr34 equivalent. Coding region starts at nucleotide 2426 and ends at nucleotide 14212.
SEQ ID NO:7—EST of *Triticum aestivum* Lr34 (GenBank Accession No. CJ669561).
SEQ ID NO:8—EST of *Triticum aestivum* Lr34 (GenBank Accession No. DR733734).
SEQ ID NO:9—EST of *Triticum aestivum* Lr34 (GenBank Accession No. CJ562397).
SEQ ID NO:10—EST of *Triticum aestivum* Lr34 (GenBank Accession No. CV773074).
SEQ ID NO:11—EST for *Hordeum vulgare* Lr34 (GenBank Accession No. BU991506).
SEQ ID NO's: 12-46—Oligonucleotide primers.
SEQ ID NO:47—Rice ABC transporter PDR23.
SEQ ID NO:48—*Arabidopsis thaliana* ABC transporter PDR5.
SEQ ID NO:49—*Arabidopsis thaliana* ABC transporter PDR9.
SEQ ID NO: 50—N-terminal Walker A sequence of Lr34.
SEQ ID NO: 51—C-terminal Walker A sequence of Lr34.
SEQ ID NO: 52—N-terminal ABC signature sequence of Lr34.
SEQ ID NO: 53—C-terminal ABC signature sequence of Lr34.
SEQ ID NO: 54—N-terminal Walker B sequence of Lr34.
SEQ ID NO: 55—C-terminal Walker B sequence of Lr34.
SEQ ID NO: 56—Consensus Walker A sequence of ABC transporters.
SEQ ID NO: 57—Consensus Walker B sequence of ABC transporters.
SEQ ID NO: 58—Consensus ABC signature sequence of ABC transporters.
SEQ ID NO:59—PDR signature sequence 1.
SEQ ID NO:60—PDR signature sequence 2.
SEQ ID NO:61—PDR signature sequence 3.
SEQ ID NO:62—PDR signature sequence 4.
SEQ ID NO:63—Polypeptide encoded by Lr34 homeolog on wheat chromosome 7B.
SEQ ID NO:64—Open reading frame encoding Lr34 homeolog on wheat chromosome 7B.

DETAILED DESCRIPTION OF THE INVENTION

General Techniques

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, plant molecular biology, protein chemistry, and biochemistry).

Unless otherwise indicated, the recombinant protein, cell culture, and immunological techniques utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T. A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D. M. Glover and B. D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F. M. Ausubel et al. (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory, (1988), and J. E. Coligan et al. (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present).

Polypeptides/Peptides

By "substantially purified polypeptide" or "purified polypeptide" we mean a polypeptide that has generally been

separated from the lipids, nucleic acids, other peptides, and other contaminating molecules with which it is associated in its native state. Preferably, the substantially purified polypeptide is at least 90% free from other components with which it is naturally associated.

The term "recombinant" in the context of a polypeptide refers to the polypeptide when produced by a cell, or in a cell-free expression system, in an altered amount or at an altered rate compared to its native state. In one embodiment, the cell is a cell that does not naturally produce the polypeptide. However, the cell may be a cell which comprises a non-endogenous gene that causes an altered amount of the polypeptide to be produced. A recombinant polypeptide of the invention includes polypeptides which have not been separated from other components of the transgenic (recombinant) cell, or cell-free expression system, in which it is produced, and polypeptides produced in such cells or cell-free systems which are subsequently purified away from at least some other components. In an embodiment, a "recombinant polypeptide" is a polypeptide made by the expression of a recombinant polynucleotide in a cell, preferably a plant cell and more preferably a cereal plant cell.

The terms "polypeptide" and "protein" are generally used interchangeably.

As used herein, the term "adult plant pathogen resistance polypeptide" refers to a protein encoded by a gene which ordinarily confers upon an adult plant an enhanced resistance to a plant pathogen when compared to an isogenic plant lacking said gene, and which confers on seedlings of the same plant substantially less or no resistance to the same pathogen when the plant is grown in normal field conditions. This term also refers to the naturally produced protein (or wild type protein from which a mutant protein is derived) encoded by a gene conferring upon an adult plant (for example, of the wheat cultivar Frontana), but not a seedling, when grown in normal field conditions, enhanced resistance to a plant pathogen. Typically, adult plant pathogen resistance polypeptides do not confer a hypersensitive response on the plants in the presence of the pathogen, and the resistance is durable in the field over time. As used herein, "adult plant" refers to a plant that has commenced the reproductive phase of growth and development. In an embodiment, less than half of the protein is produced per gram dry weight in leaves of a seedling when compared to leaves of the adult plant. Examples of plant pathogens for which resistance is enhanced include, but are not limited to, *Fusarium graminearum, Erysiphe graminis f.* sp. *tritici, Bipolaris sorokiniana, Puccinia graminis f.* sp. *tritici, Puccinia striiformis* and *Puccinia recondite f.* sp. *tritici.*

The % identity of a polypeptide is determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. The query sequence is at least 150 amino acids in length, and the GAP analysis aligns the two sequences over a region of at least 150 amino acids. More preferably, the query sequence is at least 500 amino acids in length, and the GAP analysis aligns the two sequences over a region of at least 500 amino acids. More preferably, the query sequence is at least 1,000 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 1,000 amino acids. Even more preferably, the query sequence is at least 1,250 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 1,250 amino acids. Even more preferably, the GAP analysis aligns two sequences over their entire length.

As used herein a "biologically active" fragment is a portion of a polypeptide of the invention which maintains a defined activity of the full-length polypeptide. Biologically active fragments can be any size as long as they maintain the defined activity but are preferably at least 1000 or at least 1200 amino acid residues long. Preferably, the biologically active fragment maintains at least 10% of the activity of the full length protein.

The phrase "enhanced resistance to a plant pathogen" is used herein as a relative term such that a plant of the invention has an increased level of resistance to a plant pathogen when compared to a genetically identical lacking the exogenous polynucleotide. Enhanced resistance can be determined by a number of methods known in the art such as analysing the plants for the amount of pathogen and/or analysing plant growth or the amount of damage to a plant in the presence of the pathogen.

As used herein, the term "has accelerated senescence of flag leaf tips" refers to an early onset of aging of the extremity of the lowermost leaf on the stem of a plant. This is used herein as a relative term such that a plant of the invention has an increased senescence of flag leaf tips when compared to a genetically identical flag leaf lacking the exogenous polynucleotide. Accelerated senescence of flag leaf tips can be measured by any means known in the art, such as that described in Example 5.

With regard to a defined polypeptide, it will be appreciated that % identity figures higher than those provided above will encompass preferred embodiments. Thus, where applicable, in light of the minimum % identity figures, it is preferred that the polypeptide comprises an amino acid sequence which is at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 76%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to the relevant nominated SEQ ID NO.

As used herein, the phrase "at a position corresponding to amino acid number" or variations thereof refers to the relative position of the amino acid compared to surrounding amino acids. In this regard, in some embodiments a polypeptide of the invention may have deletional or substitutional mutations which alters the relative positioning of the amino acid when aligned against, for instance, SEQ ID NO:1 and/or SEQ ID NO:4. For example, the polypeptide with a sequence as provided in SEQ ID NO:1 has a single amino acid deletion when compared to the polypeptide with a sequence as provided in SEQ ID NO:4, namely the phenylalanine at position number 546 of SEQ ID NO:4 is missing in SEQ ID NO:1 and has not be substituted with another amino acid. As a result, the skilled person will appreciate that amino acid number 634 of SEQ ID NO:4 (Y) corresponds to amino acid number 633 of SEQ ID NO:4 (H).

Amino acid sequence mutants of the polypeptides of the present invention can be prepared by introducing appropriate nucleotide changes into a nucleic acid of the present invention, or by in vitro synthesis of the desired polypeptide. Such mutants include, for example, deletions, insertions or substitutions of residues within the amino acid sequence. A combination of deletion, insertion and substitution can be made to arrive at the final construct, provided that the final peptide

product possesses the desired characteristics. Preferred amino acid sequence mutants have only one, two, three, four or less than 10 amino acid changes relative to the reference wildtype polypeptide.

Mutant (altered) peptides can be prepared using any technique known in the art. For example, a polynucleotide of the invention can be subjected to in vitro mutagenesis. Such in vitro mutagenesis techniques include sub-cloning the polynucleotide into a suitable vector, transforming the vector into a "mutator" strain such as the *E. coli* XL-1 red (Stratagene) and propagating the transformed bacteria for a suitable number of generations. In another example, the polynucleotides of the invention are subjected to DNA shuffling techniques as broadly described by Harayama (1998). Products derived from mutated/altered DNA can readily be screened using techniques described herein to determine if they possess pathogen resistance and/or ABC transporter activity.

In designing amino acid sequence mutants, the location of the mutation site and the nature of the mutation will depend on characteristic(s) to be modified. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting other residues adjacent to the located site.

Amino acid sequence deletions generally range from about 1 to 15 residues, more preferably about 1 to 10 residues and typically about 1 to 5 contiguous residues.

Substitution mutants have at least one amino acid residue in the polypeptide molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s). Other sites of interest are those in which particular residues obtained from various strains or species are identical. These positions may be important for biological activity. These sites, especially those falling within a sequence of at least three other identically conserved sites, are preferably substituted in a relatively conservative manner. Such conservative substitutions are shown in Table 1 under the heading of "exemplary substitutions".

In a preferred embodiment a mutant/variant polypeptide has one or two or three or four conservative amino acid changes when compared to a naturally occurring polypeptide. Details of conservative amino acid changes are provided in Table 1. In a preferred embodiment, the changes are not in one or more of the motifs which are highly conserved between the different polypeptides provided herewith. As the skilled person would be aware, such minor changes can reasonably be predicted not to alter the activity of the polypeptide when expressed in a recombinant cell.

TABLE 1

Exemplary substitutions.

Original Residue	Exemplary Substitutions
Ala (A)	val; leu; ile; gly
Arg (R)	lys
Asn (N)	gln; his
Asp (D)	glu
Cys (C)	ser
Gln (Q)	asn; his
Glu (E)	asp
Gly (G)	pro, ala
His (H)	asn; gln
Ile (I)	leu; val; ala
Leu (L)	ile; val; met; ala; phe

TABLE 1-continued

Exemplary substitutions.

Original Residue	Exemplary Substitutions
Lys (K)	arg
Met (M)	leu; phe
Phe (F)	leu; val; ala
Pro (P)	gly
Ser (S)	thr
Thr (T)	ser
Trp (W)	tyr
Tyr (Y)	trp; phe
Val (V)	ile; leu; met; phe, ala

In an embodiment, the protein of the invention is a PDR (pleiotropic drug resistance homolog) ABC transporter and comprises two nucleotide binding domains (NBD) and two transmembrane domains configured as shown in FIG. **5**.

The primary amino acid sequence of Lr34 can be used to design variants/mutants thereof based on comparisons with closely related ABC transporters. As the skilled addressee will appreciate, residues highly conserved amongst closely related PDR ABC transporters are less likely to be able to be altered, especially with non-conservative substitutions, and activity maintained than less conserved residues. Such conserved regions and possible substitutions are described by Rae (2007), van den Brule and Smart (2002) and Verrier et al. (2008). The polypeptide generally comprises two Walker A boxes ($GX_4GK[ST]$) (SEQ ID NO:56) (corresponds to SEQ ID NO's: 50 and 51 of Lr34) and two Walker B boxes ($(hydrophobic)_4[DE]$) (SEQ ID NO:57) (corresponds to SEQ ID NO's: 54 and 55 of Lr34), and two ABC signature motifs ($[LIVMFY]S[SGM][GE]X_3[RKA][LIVMYA]X[LIVFMT][AG]$) (SEQ ID NO:58) (corresponds to SEQ ID NO's: 52 and 53 of Lr34), with each NBD comprising, in order from the N-terminus, a Walker A, ABC signature and Walker B motif (see, for example, FIG. **4**). In the above sequences X may be any amino acid, and may be independently the same or different.

Furthermore, the polypeptide generally comprises a PDR signature 1 (LLLGPP) (SEQ ID NO:59) which is immediately N-terminal to and slightly overlaps with the N-terminal Walker A box; PDR signature 2 (GLDSST) (SEQ ID NO:60) which starts about four residues C-terminal to the N-terminal Walker B box; PDR signature 3 (GLD[AT]R[AS]AAIV[MI]R) (SEQ ID NO:61) which starts about four residues C-terminal to the C-terminal Walker B box; and PDR signature 4 (VCTIHQPS) (SEQ ID NO:62) which starts about 86 residues C-terminal to PDR signature 3.

In an embodiment, the polypeptide of the invention comprises one or more of the amino acids motifs provided as SEQ ID NO's: 56 to 58, preferably two copies of all three. More preferably, the polypeptide of the invention comprises one or more of the amino acids motifs provided as SEQ ID NO's: 50 to 55, preferably all six.

In addition, in yet a further embodiment the polypeptide of the invention comprises one or more of the amino acids motifs provided as SEQ ID NO's: 59 to 62, preferably all four.

Sources of naturally occurring variants of SEQ ID NO:1 which confer resistance as described herein are outlined in Table 5. Based on the information provided herein, the skilled person could readily determine the amino acid sequence of these naturally occurring variants, as well as polynucleotides encoding therefor.

Also included within the scope of the invention are polypeptides of the present invention which are differentially

modified during or after synthesis, e.g., by biotinylation, benzylation, glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. The polypeptides may be post-translationally modified in a cell, for example by phosphorylation, which may modulate its activity. These modifications may serve to increase the stability and/or bioactivity of the polypeptide of the invention.

Polypeptides of the present invention can be produced in a variety of ways, including production and recovery of natural polypeptides, production and recovery of recombinant polypeptides, and chemical synthesis of the polypeptides. In one embodiment, an isolated polypeptide of the present invention is produced by culturing a cell capable of expressing the polypeptide under conditions effective to produce the polypeptide, and recovering the polypeptide. A preferred cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit polypeptide production. An effective medium refers to any medium in which a cell is cultured to produce a polypeptide of the present invention. Such medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art. A preferred means of producing the polypeptides is in a transgenic plant, preferably a transgenic cereal plant.

Polynucleotides and Genes

The present invention refers to various polynucleotides. As used herein, a "polynucleotide" or "nucleic acid" or "nucleic acid molecule" means a polymer of nucleotides, which may be DNA or RNA or a combination thereof, and includes mRNA, cRNA, cDNA, tRNA, siRNA, shRNA and hpRNA. It may be DNA or RNA of cellular, genomic or synthetic origin, for example made on an automated synthesizer, and may be combined with carbohydrate, lipids, protein or other materials, labelled with fluorescent or other groups, or attached to a solid support to perform a particular activity defined herein, or comprise one or more modified nucleotides not found in nature, well known to those skilled in the art. The polymer may be single-stranded, essentially double-stranded or partly double-stranded. An example of a partly-double stranded RNA molecule is a hairpin RNA (hpRNA), short hairpin RNA (shRNA) or self-complementary RNA which include a double stranded stem formed by basepairing between a nucleotide sequence and its complement and a loop sequence which covalently joins the nucleotide sequence and its complement. Basepairing as used herein refers to standard basepairing between nucleotides, including G: U basepairs. "Complementary" means two polynucleotides are capable of basepairing (hybridizing) along part of their lengths, or along the full length of one or both. A "hybridized polynucleotide" means the polynucleotide is actually basepaired to its complement. The term "polynucleotide" is used interchangeably herein with the term "nucleic acid".

By "isolated polynucleotide" we mean a polynucleotide which has generally been separated from the polynucleotide sequences with which it is associated or linked in its native state. Preferably, the isolated polynucleotide is at least 90% free from other components with which it is naturally associated.

The present invention involves modification of gene activity and the construction and use of chimeric genes. As used herein, the term "gene" includes any deoxyribonucleotide sequence which includes a protein coding region or which is transcribed in a cell but not translated, as well as associated non-coding and regulatory regions. Such associated regions are typically located adjacent to the coding region or the transcribed region on both the 5' and 3' ends for a distance of about 2 kb on either side. In this regard, the gene may include control signals such as promoters, enhancers, termination and/or polyadenylation signals that are naturally associated with a given gene, or heterologous control signals in which case the gene is referred to as a "chimeric gene". The sequences which are located 5' of the coding region and which are present on the mRNA are referred to as 5' non-translated sequences. The sequences which are located 3' or downstream of the coding region and which are present on the mRNA are referred to as 3' non-translated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene.

A "Lr34 gene" as used herein refers to a nucleotide sequence which is homologous to the isolated Lr34 gene (SEQ ID NO:3) or Lr34 cDNA (SEQ ID NO:2) described herein, which encodes a protein that confers resistance to a pathogen, preferably a fungal pathogen, on a plant, preferably a cereal plant and more preferably a wheat plant. Preferably, the protein confers resistance to more than one fungal pathogen. Lr34 genes include the naturally occurring alleles or variants existing in cereals such as wheat, including those encoded by the D genomes of hexaploid wheat and its D genome diploid progenitors or relatives, as well as non-naturally occurring variants which may be produced by those skilled in the art of gene modification. Nucleic acid molecules having the nucleotide sequence shown herein as SEQ ID NO:2 (cDNA) or SEQ ID NO:3 (genomic sequence), encoding a protein with amino acid sequence SEQ ID NO:1, are examples of an Lr34 gene. In a preferred embodiment, a Lr34 gene refers to a nucleic acid molecule comprising nucleotides having a sequence having at least 90% identity to SEQ ID NO:2.

A genomic form or clone of a gene containing the transcribed region may be interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." An "intron" as used herein is a segment of a gene which is transcribed as part of a primary RNA transcript but is not present in the mature mRNA molecule. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA). Introns may contain regulatory elements such as enhancers. "Exons" as used herein refer to the DNA regions corresponding to the RNA sequences which are present in the mature mRNA or the mature RNA molecule in cases where the RNA molecule is not translated. An mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide. The term "gene" includes a synthetic or fusion molecule encoding all or part of the proteins of the invention described herein and a complementary nucleotide sequence to any one of the above. A gene may be introduced into an appropriate vector for extrachromosomal maintenance in a cell or for integration into the host genome.

As used herein, a "chimeric gene" refers to any gene that is not a native gene in its native location. Typically, a chimeric gene comprises regulatory and transcribed or protein coding sequences that are not found together in nature. Accordingly,

a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. The term "endogenous" is used herein to refer to a substance that is normally present or produced in an unmodified plant at the same developmental stage as the plant under investigation. An "endogenous gene" refers to a native gene in its natural location in the genome of an organism. As used herein, "recombinant nucleic acid molecule", "recombinant polynucleotide" or variations thereof refer to a nucleic acid molecule which has been constructed or modified by recombinant DNA technology. The terms "foreign polynucleotide" or "exogenous polynucleotide" or "heterologous polynucleotide" and the like refer to any nucleic acid which is introduced into the genome of a cell by experimental manipulations. For example, the present inventors have identified the Lr34 homeolog on wheat chromosome 7B (see SEQ ID NO's 63 and 64). The skilled person can use this information to mutant the Lr34 gene homeolog in durum wheat such that it encodes a protein of the invention which lacks a phenylalanine residue or any amino acid at a position corresponding to amino acid number 546 of SEQ ID NO:4, and has an amino acid other than a tyrosine residue at a position corresponding to amino acid number 634 of SEQ ID NO:4. Such a mutated gene, and the encoded mRNA, would be considered as an "exogenous" polynucleotide of the invention.

Foreign or exogenous genes may be genes that are inserted into a non-native organism, native genes introduced into a new location within the native host, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure. The term "genetically modified" includes introducing genes into cells by transformation or transduction, mutating genes in cells and altering or modulating the regulation of a gene in a cell or organisms to which these acts have been done or their progeny.

Furthermore, the term "exogenous" in the context of a polynucleotide (nucleic acid) refers to the polynucleotide when present in a cell, or in a cell-free expression system, in an altered amount compared to its native state. In one embodiment, the cell is a cell that does not naturally comprise the polynucleotide. However, the cell may be a cell which comprises a non-endogenous polynucleotide resulting in an altered amount of production of the encoded polypeptide. An exogenous polynucleotide of the invention includes polynucleotides which have not been separated from other components of the transgenic (recombinant) cell, or cell-free expression system, in which it is present, and polynucleotides produced in such cells or cell-free systems which are subsequently purified away from at least some other components. The exogenous polynucleotide (nucleic acid) can be a contiguous stretch of nucleotides existing in nature, or comprise two or more contiguous stretches of nucleotides from different sources (naturally occurring and/or synthetic) joined to form a single polynucleotide. Typically such chimeric polynucleotides comprise at least an open reading frame encoding a polypeptide of the invention operably linked to a promoter suitable of driving transcription of the open reading frame in a cell of interest.

The % identity of a polynucleotide is determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. The query sequence is at least 450 nucleotides in length, and the GAP analysis aligns the two sequences over a region of at least 450 nucleotides. Preferably, the query sequence is at least 1,500 nucleotides in length, and the GAP analysis aligns the two sequences over a region of at least 1,500 nucleotides. Even more preferably, the query sequence is at least 3,000 nucleotides in length and the GAP analysis aligns the two sequences over a region of at least 3,000 nucleotides. Even more preferably, the GAP analysis aligns two sequences over their entire length.

With regard to the defined polynucleotides, it will be appreciated that % identity figures higher than those provided above will encompass preferred embodiments. Thus, where applicable, in light of the minimum % identity figures, it is preferred that the polynucleotide comprises a polynucleotide sequence which is at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to the relevant nominated SEQ ID NO.

In a preferred embodiment, the polynucleotide of the invention is not a sequence of nucleotides as provided in any one of SEQ ID NO's 7 to 11.

In a further embodiment, the present invention relates to polynucleotides which are substantially identical to those specifically described herein. As used herein, with reference to a polynucleotide the term "substantially identical" means the substitution of one or a few (for example 2, 3, or 4) nucleotides whilst maintaining at least one activity of the native protein encoded by the polynucleotide. In addition, this term includes the addition or deletion of nucleotides which results in the increase or decrease in size of the encoded native protein by one or a few (for example 2, 3, or 4) amino acids whilst maintaining at least one activity of the native protein encoded by the polynucleotide.

The present invention refers to use of oligonucleotides. As used herein, "oligonucleotides" are polynucleotides up to 50 nucleotides in length. The minimum size of such oligonucleotides is the size required for the formation of a stable hybrid between an oligonucleotide and a complementary sequence on a nucleic acid molecule of the present invention. They can be RNA, DNA, or combinations or derivatives of either. Oligonucleotides are typically relatively short single stranded molecules of 10 to 30 nucleotides, commonly 15-25 nucleotides in length. When used as a probe or as a primer in an amplification reaction, the minimum size of such an oligonucleotide is the size required for the formation of a stable hybrid between the oligonucleotide and a complementary sequence on a target nucleic acid molecule. Preferably, the oligonucleotides are at least 15 nucleotides, more preferably at least 18 nucleotides, more preferably at least 19 nucleotides, more preferably at least 20 nucleotides, even more preferably at least 25 nucleotides in length. Oligonucleotides of the present invention used as a probe are typically conjugated with a label such as a radioisotope, an enzyme, biotin, a fluorescent molecule or a chemiluminescent molecule.

The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic acid molecules, or primers to produce nucleic acid molecules. Probes and/or primers can be used to clone homologues of the polynucleotides of the invention from other species. Furthermore, hybridization techniques known in the art can also be used to screen genomic or cDNA libraries for such homologues.

Polynucleotides and oligonucleotides of the present invention include those which hybridize under stringent conditions to a sequence provided as SEQ ID NO's: 2 and/or 3. As used herein, stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% $NaDodSO_4$ at 50° C.; (2) employ during hybridisation a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42° C.; or (3) employ 50% formamide, 5×SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5×Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS and 10% dextran sulfate at 42° C. in 0.2×SSC and 0.1% SDS.

Polynucleotides of the present invention may possess, when compared to naturally occurring molecules, one or more mutations which are deletions, insertions, or substitutions of nucleotide residues. Mutants can be either naturally occurring (that is to say, isolated from a natural source) or synthetic (for example, by performing site-directed mutagenesis on the nucleic acid). A variant of a polynucleotide or an oligonucleotide of the invention includes molecules of varying sizes of, and/or are capable of hybridising to, the wheat genome close to that of the reference polynucleotide or oligonucleotide molecules defined herein. For example, variants may comprise additional nucleotides (such as 1, 2, 3, 4, or more), or less nucleotides as long as they still hybridise to the target region. Furthermore, a few nucleotides may be substituted without influencing the ability of the oligonucleotide to hybridise to the target region. In addition, variants may readily be designed which hybridise close to, for example to within 50 nucleotides, the region of the plant genome where the specific oligonucleotides defined herein hybridise. In particular, this includes polynucleotides which encode the same polypeptide or amino acid sequence but which vary in nucleotide sequence by redundancy of the genetic code. The terms "polynucleotide variant" and "variant" also include naturally occurring allelic variants.

Nucleic Acid Constructs

The present invention includes nucleic acid constructs comprising the polynucleotides of the invention, and vectors and host cells containing these, methods of their production and use, and uses thereof. The present invention refers to elements which are operably connected or linked. "Operably connected" or "operably linked" and the like refer to a linkage of polynucleotide elements in a functional relationship. Typically, operably connected nucleic acid sequences are contiguously linked and, where necessary to join two protein coding regions, contiguous and in reading frame. A coding sequence is "operably connected to" another coding sequence when RNA polymerase will transcribe the two coding sequences into a single RNA, which if translated is then translated into a single polypeptide having amino acids derived from both coding sequences. The coding sequences need not be contiguous to one another so long as the expressed sequences are ultimately processed to produce the desired protein.

As used herein, the term "cis-acting sequence", "cis-acting element" or "cis-regulatory region" or "regulatory region" or similar term shall be taken to mean any sequence of nucleotides, which when positioned appropriately and connected relative to an expressible genetic sequence, is capable of regulating, at least in part, the expression of the genetic sequence. Those skilled in the art will be aware that a cis-regulatory region may be capable of activating, silencing, enhancing, repressing or otherwise altering the level of expression and/or cell-type-specificity and/or developmental specificity of a gene sequence at the transcriptional or post-transcriptional level. In preferred embodiments of the present invention, the cis-acting sequence is an activator sequence that enhances or stimulates the expression of an expressible genetic sequence.

"Operably connecting" a promoter or enhancer element to a transcribable polynucleotide means placing the transcribable polynucleotide (e.g., protein-encoding polynucleotide or other transcript) under the regulatory control of a promoter, which then controls the transcription of that polynucleotide. In the construction of heterologous promoter/structural gene combinations, it is generally preferred to position a promoter or variant thereof at a distance from the transcription start site of the transcribable polynucleotide which is approximately the same as the distance between that promoter and the protein coding region it controls in its natural setting; i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of function. Similarly, the preferred positioning of a regulatory sequence element (e.g., an operator, enhancer etc) with respect to a transcribable polynucleotide to be placed under its control is defined by the positioning of the element in its natural setting; i.e., the genes from which it is derived.

"Promoter" or "promoter sequence" as used herein refers to a region of a gene, generally upstream (5') of the RNA encoding region, which controls the initiation and level of transcription in the cell of interest. A "promoter" includes the transcriptional regulatory sequences of a classical genomic gene, such as a TATA box and CCAAT box sequences, as well as additional regulatory elements (i.e., upstream activating sequences, enhancers and silencers) that alter gene expression in response to developmental and/or environmental stimuli, or in a tissue-specific or cell-type-specific manner. A promoter is usually, but not necessarily (for example, some PolIII promoters), positioned upstream of a structural gene, the expression of which it regulates. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene. Promoters may contain additional specific regulatory elements, located more distal to the start site to further enhance expression in a cell, and/or to alter the timing or inducibility of expression of a structural gene to which it is operably connected.

"Constitutive promoter" refers to a promoter that directs expression of an operably linked transcribed sequence in many or all tissues of an organism such as a plant. The term constitutive as used herein does not necessarily indicate that a gene is expressed at the same level in all cell types, but that the gene is expressed in a wide range of cell types, although some variation in level is often detectable. "Selective expression" as used herein refers to expression almost exclusively in specific organs of, for example, the plant, such as, for example, endosperm, embryo, leaves, fruit, tubers or root. In a preferred embodiment, a promoter is expressed selectively or preferentially in leaves and/or stems of a plant, preferably a cereal plant. Selective expression may therefore be contrasted with constitutive expression, which refers to expression in many or all tissues of a plant under most or all of the conditions experienced by the plant.

Selective expression may also result in compartmentation of the products of gene expression in specific plant tissues, organs or developmental stages. Compartmentation in specific subcellular locations such as the plastid, cytosol, vacuole, or apoplastic space may be achieved by the inclusion in the structure of the gene product of appropriate signals, eg. a

signal peptide, for transport to the required cellular compartment, or in the case of the semi-autonomous organelles (plastids and mitochondria) by integration of the transgene with appropriate regulatory sequences directly into the organelle genome.

A "tissue-specific promoter" or "organ-specific promoter" is a promoter that is preferentially expressed in one tissue or organ relative to many other tissues or organs, preferably most if not all other tissues or organs in, for example, a plant. Typically, the promoter is expressed at a level 10-fold higher in the specific tissue or organ than in other tissues or organs.

The promoters contemplated by the present invention may be native to the host plant to be transformed or may be derived from an alternative source, where the region is functional in the host plant. Other sources include the *Agrobacterium* T-DNA genes, such as the promoters of genes for the biosynthesis of nopaline, octapine, mannopine, or other opine promoters, tissue specific promoters (see, e.g., U.S. Pat. No. 5,459,252 and WO 91/13992); promoters from viruses (including host specific viruses), or partially or wholly synthetic promoters. Numerous promoters that are functional in mono- and dicotyledonous plants are well known in the art (see, for example, Greve, 1983; Salomon et al., 1984; Garfinkel et al., 1983; Barker et al., 1983); including various promoters isolated from plants and viruses such as the cauliflower mosaic virus promoter (CaMV 35S, 19S). Non-limiting methods for assessing promoter activity are disclosed by Medberry et al. (1992, 1993), Sambrook et al. (1989, supra) and U.S. Pat. No. 5,164,316.

Alternatively or additionally, the promoter may be an inducible promoter or a developmentally regulated promoter which is capable of driving expression of the introduced polynucleotide at an appropriate developmental stage of the, for example, plant. Other cis-acting sequences which may be employed include transcriptional and/or translational enhancers. Enhancer regions are well known to persons skilled in the art, and can include an ATG translational initiation codon and adjacent sequences. When included, the initiation codon should be in phase with the reading frame of the coding sequence relating to the foreign or exogenous polynucleotide to ensure translation of the entire sequence if it is to be translated. Translational initiation regions may be provided from the source of the transcriptional initiation region, or from a foreign or exogenous polynucleotide. The sequence can also be derived from the source of the promoter selected to drive transcription, and can be specifically modified so as to increase translation of the mRNA.

In an embodiment, the promoter is at least capable of expressing the polypeptide in leaves of the plant, particularly adult leaves. Examples of leaf-specific promoters which can be used include those described in Yamamoto et al. (1994 and 1997), Kwon et al. (1994), Gotor et al. (1993), Orozco et al. (1993), Matsuoka et al. (1993) and Stockhaus et al. (1987 and 1989).

The nucleic acid construct of the present invention may comprise a 3' non-translated sequence from about 50 to 1,000 nucleotide base pairs which may include a transcription termination sequence. A 3' non-translated sequence may contain a transcription termination signal which may or may not include a polyadenylation signal and any other regulatory signals capable of effecting mRNA processing. A polyadenylation signal functions for addition of polyadenylic acid tracts to the 3' end of a mRNA precursor. Polyadenylation signals are commonly recognized by the presence of homology to the canonical form 5' AATAAA-3' although variations are not uncommon. Transcription termination sequences which do not include a polyadenylation signal include terminators for PolI or PolIII RNA polymerase which comprise a run of four or more thymidines. Examples of suitable 3' non-translated sequences are the 3' transcribed non-translated regions containing a polyadenylation signal from an octopine synthase (ocs) gene or nopaline synthase (nos) gene of *Agrobacterium tumefaciens* (Bevan et al., 1983). Suitable 3' non-translated sequences may also be derived from plant genes such as the ribulose-1,5-bisphosphate carboxylase (ssRUBISCO) gene, although other 3' elements known to those of skill in the art can also be employed.

As the DNA sequence inserted between the transcription initiation site and the start of the coding sequence, i.e., the untranslated 5' leader sequence (5'UTR), can influence gene expression if it is translated as well as transcribed, one can also employ a particular leader sequence. Suitable leader sequences include those that comprise sequences selected to direct optimum expression of the foreign or endogenous DNA sequence. For example, such leader sequences include a preferred consensus sequence which can increase or maintain mRNA stability and prevent inappropriate initiation of translation as for example described by Joshi (1987).

Vectors

The present invention includes use of vectors for manipulation or transfer of genetic constructs. By "chimeric vector" is meant a nucleic acid molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage, or plant virus, into which a nucleic acid sequence may be inserted or cloned. A vector preferably is double-stranded DNA and contains one or more unique restriction sites and may be capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or capable of integration into the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector may be an autonomously replicating vector, i.e., a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g., a linear or closed circular plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into a cell, is integrated into the genome of the recipient cell and replicated together with the chromosome(s) into which it has been integrated. A vector system may comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the cell into which the vector is to be introduced. The vector may also include a selection marker such as an antibiotic resistance gene, a herbicide resistance gene or other gene that can be used for selection of suitable transformants. Examples of such genes are well known to those of skill in the art.

The nucleic acid construct of the invention can be introduced into a vector, such as a plasmid. Plasmid vectors typically include additional nucleic acid sequences that provide for easy selection, amplification, and transformation of the expression cassette in prokaryotic and eukaryotic cells, e.g., pUC-derived vectors, pSK-derived vectors, pGEM-derived vectors, pSP-derived vectors, pBS-derived vectors, or binary vectors containing one or more T-DNA regions. Additional nucleic acid sequences include origins of replication to provide for autonomous replication of the vector, selectable marker genes, preferably encoding antibiotic or herbicide resistance, unique multiple cloning sites providing for multiple sites to insert nucleic acid sequences or genes encoded in

the nucleic acid construct, and sequences that enhance transformation of prokaryotic and eukaryotic (especially plant) cells.

By "marker gene" is meant a gene that imparts a distinct phenotype to cells expressing the marker gene and thus allows such transformed cells to be distinguished from cells that do not have the marker. A selectable marker gene confers a trait for which one can "select" based on resistance to a selective agent (e.g., a herbicide, antibiotic, radiation, heat, or other treatment damaging to untransformed cells). A screenable marker gene (or reporter gene) confers a trait that one can identify through observation or testing, i.e., by "screening" (e.g., β-glucuronidase, luciferase, GFP or other enzyme activity not present in untransformed cells). The marker gene and the nucleotide sequence of interest do not have to be linked.

To facilitate identification of transformants, the nucleic acid construct desirably comprises a selectable or screenable marker gene as, or in addition to, the foreign or exogenous polynucleotide. The actual choice of a marker is not crucial as long as it is functional (i.e., selective) in combination with the plant cells of choice. The marker gene and the foreign or exogenous polynucleotide of interest do not have to be linked, since co-transformation of unlinked genes as, for example, described in U.S. Pat. No. 4,399,216 is also an efficient process in plant transformation.

Examples of bacterial selectable markers are markers that confer antibiotic resistance such as ampicillin, erythromycin, chloramphenicol or tetracycline resistance, preferably kanamycin resistance. Exemplary selectable markers for selection of plant transformants include, but are not limited to, a hyg gene which encodes hygromycin B resistance; a neomycin phosphotransferase (nptII) gene conferring resistance to kanamycin, paromomycin, G418; a glutathione-S-transferase gene from rat liver conferring resistance to glutathione derived herbicides as, for example, described in EP 256223; a glutamine synthetase gene conferring, upon overexpression, resistance to glutamine synthetase inhibitors such as phosphinothricin as, for example, described in WO 87/05327, an acetyltransferase gene from *Streptomyces viridochromogenes* conferring resistance to the selective agent phosphinothricin as, for example, described in EP 275957, a gene encoding a 5-enolshikimate-3-phosphate synthase (EPSPS) conferring tolerance to N-phosphonomethylglycine as, for example, described by Hinchee et al. (1988), a bar gene conferring resistance against bialaphos as, for example, described in WO91/02071; a nitrilase gene such as bxn from *Klebsiella ozaenae* which confers resistance to bromoxynil (Stalker et al., 1988); a dihydrofolate reductase (DHFR) gene conferring resistance to methotrexate (Thillet et al., 1988); a mutant acetolactate synthase gene (ALS), which confers resistance to imidazolinone, sulfonylurea or other ALS-inhibiting chemicals (EP 154,204); a mutated anthranilate synthase gene that confers resistance to 5-methyl tryptophan; or a dalapon dehalogenase gene that confers resistance to the herbicide.

Preferred screenable markers include, but are not limited to, a uidA gene encoding a β-glucuronidase (GUS) enzyme for which various chromogenic substrates are known, a β-galactosidase gene encoding an enzyme for which chromogenic substrates are known, an aequorin gene (Prasher et al., 1985), which may be employed in calcium-sensitive bioluminescence detection; a green fluorescent protein gene (Niedz et al., 1995) or derivatives thereof; a luciferase (luc) gene (Ow et al., 1986), which allows for bioluminescence detection, and others known in the art. By "reporter molecule" as used in the present specification is meant a molecule that, by its chemical nature, provides an analytically identifiable signal that facilitates determination of promoter activity by reference to protein product.

Preferably, the nucleic acid construct is stably incorporated into the genome of, for example, the plant. Accordingly, the nucleic acid comprises appropriate elements which allow the molecule to be incorporated into the genome, or the construct is placed in an appropriate vector which can be incorporated into a chromosome of a plant cell.

One embodiment of the present invention includes a recombinant vector, which includes at least one polynucleotide molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules of the present invention and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid.

A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels et al., Cloning Vectors: A Laboratory Manual, 1985, supp. 1987; Weissbach and Weissbach, Methods for Plant Molecular Biology, Academic Press, 1989; and Gelvin et al., Plant Molecular Biology Manual, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

The level of a protein, for example Lr34 protein, may be modulated by increasing the level of expression of a nucleotide sequence that codes for the protein in a plant cell, or decreasing the level of expression of a gene encoding the protein in the plant, leading to modified pathogen resistance. The level of expression of a gene may be modulated by altering the copy number per cell, for example by introducing a synthetic genetic construct comprising the coding sequence and a transcriptional control element that is operably connected thereto and that is functional in the cell. A plurality of transformants may be selected and screened for those with a favourable level and/or specificity of transgene expression arising from influences of endogenous sequences in the vicinity of the transgene integration site. A favourable level and pattern of transgene expression is one which results in a substantial modification of pathogen resistance or other phenotype. Alternatively, a population of mutagenized seed or a population of plants from a breeding program may be screened for individual lines with altered pathogen resistance or other phenotype associated with pathogen resistance.

Recombinant Cells

Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more recombinant molecules of the present invention, or progeny cells thereof. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption,

and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Preferred host cells are plant cells, more preferably cells of a cereal plant, more preferably barley or wheat cells, and even more preferably a wheat cell.

Transgenic Plants

The term "plant" as used herein as a noun refers to whole plants and refers to any member of the Kingdom Plantae, but as used as an adjective refers to any substance which is present in, obtained from, derived from, or related to a plant, such as for example, plant organs (e.g. leaves, stems, roots, flowers), single cells (e.g. pollen), seeds, plant cells and the like. Plantlets and germinated seeds from which roots and shoots have emerged are also included within the meaning of "plant". The term "plant parts" as used herein refers to one or more plant tissues or organs which are obtained from a plant and which comprises genomic DNA of the plant. Plant parts include vegetative structures (for example, leaves, stems), roots, floral organs/structures, seed (including embryo, cotyledons, and seed coat), plant tissue (for example, vascular tissue, ground tissue, and the like), cells and progeny of the same. The term "plant cell" as used herein refers to a cell obtained from a plant or in a plant and includes protoplasts or other cells derived from plants, gamete-producing cells, and cells which regenerate into whole plants. Plant cells may be cells in culture. By "plant tissue" is meant differentiated tissue in a plant or obtained from a plant ("explant") or undifferentiated tissue derived from immature or mature embryos, seeds, roots, shoots, fruits, tubers, pollen, tumor tissue, such as crown galls, and various forms of aggregations of plant cells in culture, such as calli. Exemplary plant tissues in or from seeds are cotyledon, embryo and embryo axis. The invention accordingly includes plants and plant parts and products comprising these, particularly seed comprising modified oil composition.

As used herein, the term "seed" refers to "mature seed" of a plant, which is either ready for harvesting or has been harvested from the plant, such as is typically harvested commercially in the field, or as "developing seed" which occurs in a plant after fertilization and prior to seed dormancy being established and before harvest.

A "transgenic plant" as used herein refers to a plant that contains a nucleic acid construct not found in a wild-type plant of the same species, variety or cultivar. That is, transgenic plants (transformed plants) contain genetic material (a transgene) that they did not contain prior to the transformation. The transgene may include genetic sequences obtained from or derived from a plant cell, or another plant cell, or a non-plant source, or a synthetic sequence. Typically, the transgene has been introduced into the plant by human manipulation such as, for example, by transformation but any method can be used as one of skill in the art recognizes. The genetic material is preferably stably integrated into the genome of the plant. The introduced genetic material may comprise sequences that naturally occur in the same species but in a rearranged order or in a different arrangement of elements, for example an antisense sequence. Plants containing such sequences are included herein in "transgenic plants". A "non-transgenic plant" is one which has not been genetically modified by the introduction of genetic material by recombinant DNA techniques. In a preferred embodiment, the transgenic plants are homozygous for each and every gene that has been introduced (transgene) so that their progeny do not segregate for the desired phenotype.

As used herein, the term "compared to an isogenic plant" refers to a plant which is isogenic relative to the transgenic plant but without the transgene of interest. Preferably, the corresponding non-transgenic plant is of the same cultivar or variety as the progenitor of the transgenic plant of interest, or a sibling plant line which lacks the construct, often termed a "segregant", or a plant of the same cultivar or variety transformed with an "empty vector" construct, and may be a non-transgenic plant. "Wild type", as used herein, refers to a cell, tissue or plant that has not been modified according to the invention. Wild-type cells, tissue or plants may be used as controls to compare levels of expression of an exogenous nucleic acid or the extent and nature of trait modification with cells, tissue or plants modified as described herein.

Transgenic plants, as defined in the context of the present invention include progeny of the plants which have been genetically modified using recombinant techniques, wherein the progeny comprise the transgene of interest. Such progeny may be obtained by self-fertilization of the primary transgenic plant or by crossing such plants with another plant of the same species. This would generally be to modulate the production of at least one protein defined herein in the desired plant or plant organ. Transgenic plant parts include all parts and cells of said plants comprising the transgene such as, for example, cultured tissues, callus and protoplasts.

Plants contemplated for use in the practice of the present invention include both monocotyledons and dicotyledons. Target plants include, but are not limited to, the following: cereals (for example, wheat, barley, rye, oats, rice, maize, sorghum and related crops); beet (sugar beet and fodder beet); pomes, stone fruit and soft fruit (apples, pears, plums, peaches, almonds, cherries, strawberries, raspberries and black-berries); leguminous plants (beans, lentils, peas, soybeans); oil plants (rape or other Brassicas, mustard, poppy, olives, sunflowers, safflower, flax, coconut, castor oil plants, cocoa beans, groundnuts); cucumber plants (marrows, cucumbers, melons); fibre plants (cotton, flax, hemp, jute); citrus fruit (oranges, lemons, grapefruit, mandarins); vegetables (spinach, lettuce, asparagus, cabbages, carrots, onions, tomatoes, potatoes, paprika); lauraceae (avocados, cinnamon, camphor); or plants such as maize, tobacco, nuts, coffee, sugar cane, tea, vines, hops, turf, bananas and natural rubber plants, as well as ornamentals (flowers, shrubs, broad-leaved trees and evergreens, such as conifers). Preferably, the plant is a cereal plant, more preferably wheat, rice, maize, triticale, oats or barley, even more preferably wheat.

As used herein, the term "wheat" refers to any species of the Genus *Triticum*, including progenitors thereof, as well as progeny thereof produced by crosses with other species. Wheat includes "hexaploid wheat" which has genome organization of AABBDD, comprised of 42 chromosomes, and "tetraploid wheat" which has genome organization of AABB, comprised of 28 chromosomes. Hexaploid wheat includes *T. aestivum, T. spelta, T. macha, T. compactum, T. sphaerococcum, T. vavilovii*, and interspecies cross thereof. A preferred species of hexaploid wheat is *T. aestivum* ssp *aestivum* (also termed "breadwheat"). Tetraploid wheat includes *T. durum* (also referred to herein as durum wheat or *Triticum turgidum* ssp. *durum*), *T. dicoccoides, T. dicoccum, T. polonicum*, and interspecies cross thereof. In addition, the term "wheat" includes potential progenitors of hexaploid or tetraploid *Triticum* sp. such as *T. uartu, T. monococcum* or *T. boeoticum* for the A genome, *Aegilops speltoides* for the B genome, and *T. tauschii* (also known as *Aegilops squamosa* or *Aegilops tauschii*) for the D genome. Particularly preferred progenitors are

those of the A genome, even more preferably the A genome progenitor is *T. monococcum*. A wheat cultivar for use in the present invention may belong to, but is not limited to, any of the above-listed species. Also encompassed are plants that are produced by conventional techniques using *Triticum* sp. as a parent in a sexual cross with a non-*Triticum* species (such as rye [*Secale cereale*]), including but not limited to *Triticale*.

As used herein, the term "barley" refers to any species of the Genus *Hordeum*, including progenitors thereof, as well as progeny thereof produced by crosses with other species. It is preferred that the plant is of a *Hordeum* species which is commercially cultivated such as, for example, a strain or cultivar or variety of *Hordeum vulgare* or suitable for commercial production of grain.

Transgenic plants, as defined in the context of the present invention include plants (as well as parts and cells of said plants) and their progeny which have been genetically modified using recombinant techniques to cause production of at least one polypeptide of the present invention in the desired plant or plant organ. Transgenic plants can be produced using techniques known in the art, such as those generally described in A. Slater et al., Plant Biotechnology—The Genetic Manipulation of Plants, Oxford University Press (2003), and P. Christou and H. Klee, Handbook of Plant Biotechnology, John Wiley and Sons (2004).

In a preferred embodiment, the transgenic plants are homozygous for each and every gene that has been introduced (transgene) so that their progeny do not segregate for the desired phenotype. The transgenic plants may also be heterozygous for the introduced transgene(s), such as, for example, in F1 progeny which have been grown from hybrid seed. Such plants may provide advantages such as hybrid vigour, well known in the art.

Four general methods for direct delivery of a gene into cells have been described: (1) chemical methods (Graham et al., 1973); (2) physical methods such as microinjection (Capecchi, 1980); electroporation (see, for example, WO 87/06614, U.S. Pat. Nos. 5,472,869, 5,384,253, WO 92/09696 and WO 93/21335); and the gene gun (see, for example, U.S. Pat. No. 4,945,050 and U.S. Pat. No. 5,141,131); (3) viral vectors (Clapp, 1993; Lu et al., 1993; Eglitis et al., 1988); and (4) receptor-mediated mechanisms (Curiel et al., 1992; Wagner et al., 1992).

Acceleration methods that may be used include, for example, microprojectile bombardment and the like. One example of a method for delivering transforming nucleic acid molecules to plant cells is microprojectile bombardment. This method has been reviewed by Yang et al., Particle Bombardment Technology for Gene Transfer, Oxford Press, Oxford, England (1994). Non-biological particles (microprojectiles) that may be coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum, and the like. A particular advantage of microprojectile bombardment, in addition to it being an effective means of reproducibly transforming monocots, is that neither the isolation of protoplasts, nor the susceptibility of *Agrobacterium* infection are required. A particle delivery system suitable for use with the present invention is the helium acceleration PDS-1000/He gun is available from Bio-Rad Laboratories. For the bombardment, immature embryos or derived target cells such as scutella or calli from immature embryos may be arranged on solid culture medium.

In another alternative embodiment, plastids can be stably transformed. Method disclosed for plastid transformation in higher plants include particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination (U.S. Pat. No. 5,451,513, U.S. Pat. No. 5,545,818, U.S. Pat. No. 5,877,402, U.S. Pat. No. 5,932,479, and WO 99/05265.

Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art (see, for example, U.S. Pat. No. 5,177,010, U.S. Pat. No. 5,104,310, U.S. Pat. No. 5,004,863, U.S. Pat. No. 5,159,135). Further, the integration of the T-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences, and intervening DNA is usually inserted into the plant genome.

Agrobacterium transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations as described (Klee et al., Plant DNA Infectious Agents, Hohn and Schell, (editors), Springer-Verlag, New York, (1985): 179-203). Moreover, technological advances in vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate construction of vectors capable of expressing various polypeptide coding genes. The vectors described have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes and are suitable for present purposes. In addition, *Agrobacterium* containing both armed and disarmed Ti genes can be used for the transformations. In those plant varieties where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single genetic locus on one chromosome. Such transgenic plants can be referred to as being hemizygous for the added gene. More preferred is a transgenic plant that is homozygous for the added structural gene; i.e., a transgenic plant that contains two added genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregant transgenic plant that contains a single added gene, germinating some of the seed produced and analyzing the resulting plants for the gene of interest.

It is also to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both exogenous genes. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation. Descriptions of other breeding methods that are commonly used for different traits and crops can be found in Fehr, Breeding Methods for Cultivar Development, J. Wilcox (editor) American Society of Agronomy, Madison Wis. (1987).

Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments. Application of these systems to different plant varieties depends upon the ability to regenerate that particular plant strain from protoplasts. Illustrative methods for the regeneration of cereals from protoplasts are described (Fujimura et al., 1985; Toriyama et al., 1986; Abdullah et al., 1986).

Other methods of cell transformation can also be used and include but are not limited to introduction of DNA into plants by direct DNA transfer into pollen, by direct injection of DNA into reproductive organs of a plant, or by direct injection of DNA into the cells of immature embryos followed by the rehydration of desiccated embryos.

The regeneration, development, and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach et al., Methods for Plant Molecular Biology, Academic Press, San Diego, (1988)). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene is well known in the art. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate regenerated plants. A transgenic plant of the present invention containing a desired exogenous nucleic acid is cultivated using methods well known to one skilled in the art.

Methods for transforming dicots, primarily by use of *Agrobacterium tumefaciens*, and obtaining transgenic plants have been published for cotton (U.S. Pat. No. 5,004,863, U.S. Pat. No. 5,159,135, U.S. Pat. No. 5,518,908); soybean (U.S. Pat. No. 5,569,834, U.S. Pat. No. 5,416,011); *Brassica* (U.S. Pat. No. 5,463,174); peanut (Cheng et al., 1996); and pea (Grant et al., 1995).

Methods for transformation of cereal plants such as wheat and barley for introducing genetic variation into the plant by introduction of an exogenous nucleic acid and for regeneration of plants from protoplasts or immature plant embryos are well known in the art, see for example, CA 2,092,588, AU 61781/94, AU 667939, U.S. Pat. No. 6,100,447, WO 97/048814, U.S. Pat. No. 5,589,617, U.S. Pat. No. 6,541,257, and other methods are set out in WO 99/14314. Preferably, transgenic wheat or barley plants are produced by *Agrobacterium tumefaciens* mediated transformation procedures. Vectors carrying the desired nucleic acid construct may be introduced into regenerable wheat cells of tissue cultured plants or explants, or suitable plant systems such as protoplasts. The regenerable wheat cells are preferably from the scutellum of immature embryos, mature embryos, callus derived from these, or the meristematic tissue.

To confirm the presence of the transgenes in transgenic cells and plants, a polymerase chain reaction (PCR) amplification or Southern blot analysis can be performed using methods known to those skilled in the art. Expression products of the transgenes can be detected in any of a variety of ways, depending upon the nature of the product, and include Western blot and enzyme assay. One particularly useful way to quantitate protein expression and to detect replication in different plant tissues is to use a reporter gene, such as GUS. Once transgenic plants have been obtained, they may be grown to produce plant tissues or parts having the desired phenotype. The plant tissue or plant parts, may be harvested, and/or the seed collected. The seed may serve as a source for growing additional plants with tissues or parts having the desired characteristics.

Marker Assisted Selection

Marker assisted selection is a well recognised method of selecting for heterozygous plants required when backcrossing with a recurrent parent in a classical breeding program. The population of plants in each backcross generation will be heterozygous for the gene of interest normally present in a 1:1 ratio in a backcross population, and the molecular marker can be used to distinguish the two alleles of the gene. By extracting DNA from, for example, young shoots and testing with a specific marker for the introgressed desirable trait, early selection of plants for further backcrossing is made whilst energy and resources are concentrated on fewer plants. To further speed up the backcrossing program, the embryo from immature seeds (25 days post anthesis) may be excised and grown up on nutrient media under sterile conditions, rather than allowing full seed maturity. This process, termed "embryo rescue", used in combination with DNA extraction at the three leaf stage and analysis of at least one Lr34 gene or allele that confers enhanced resistance to pathogens to the plant, allows rapid selection of plants carrying the desired trait, which may be nurtured to maturity in the greenhouse or field for subsequent further backcrossing to the recurrent parent.

Any molecular biological technique known in the art can be used in the methods of the present invention. Such methods include, but are not limited to, the use of nucleic acid amplification, nucleic acid sequencing, nucleic acid hybridization with suitably labeled probes, single-strand conformational analysis (SSCA), denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis (HET), chemical cleavage analysis (CCM), catalytic nucleic acid cleavage or a combination thereof (see, for example, Lemieux, 2000; Langridge et al., 2001). The invention also includes the use of molecular marker techniques to detect polymorphisms linked to alleles of (for example) Lr34 gene which confers enhanced resistance to plant pathogens. Such methods include the detection or analysis of restriction fragment length polymorphisms (RFLP), RAPD, amplified fragment length polymorphisms (AFLP) and microsatellite (simple sequence repeat, SSR) polymorphisms. The closely linked markers can be obtained readily by methods well known in the art, such as Bulked Segregant Analysis, as reviewed by Langridge et al., (2001).

The "polymerase chain reaction" ("PCR") is a reaction in which replicate copies are made of a target polynucleotide using a "pair of primers" or "set of primers" consisting of "upstream" and a "downstream" primer, and a catalyst of polymerization, such as a DNA polymerase, and typically a thermally-stable polymerase enzyme. Methods for PCR are known in the art, and are taught, for example, in "PCR" (M. J. McPherson and S. G Moller (editors), BIOS Scientific Publishers Ltd, Oxford, (2000)). PCR can be performed on cDNA obtained from reverse transcribing mRNA isolated from plant cells expressing a Lr34 gene or allele which confers enhanced resistance to plants pathogens. However, it will generally be easier if PCR is performed on genomic DNA isolated from a plant.

A primer is an oligonucleotide sequence that is capable of hybridising in a sequence specific fashion to the target sequence and being extended during the PCR. Amplicons or PCR products or PCR fragments or amplification products are extension products that comprise the primer and the newly synthesized copies of the target sequences. Multiplex PCR systems contain multiple sets of primers that result in simultaneous production of more than one amplicon. Primers may be perfectly matched to the target sequence or they may contain internal mismatched bases that can result in the introduction of restriction enzyme or catalytic nucleic acid recognition/cleavage sites in specific target sequences. Primers

may also contain additional sequences and/or contain modified or labelled nucleotides to facilitate capture or detection of amplicons. Repeated cycles of heat denaturation of the DNA, annealing of primers to their complementary sequences and extension of the annealed primers with polymerase result in exponential amplification of the target sequence. The terms target or target sequence or template refer to nucleic acid sequences which are amplified.

Methods for direct sequencing of nucleotide sequences are well known to those skilled in the art and can be found for example in Ausubel et al., (supra) and Sambrook et al., (supra). Sequencing can be carried out by any suitable method, for example, dideoxy sequencing, chemical sequencing or variations thereof. Direct sequencing has the advantage of determining variation in any base pair of a particular sequence.

TILLING

Plants of the invention can be produced using the process known as TILLING (Targeting Induced Local Lesions I N Genomes). In a first step, introduced mutations such as novel single base pair changes are induced in a population of plants by treating seeds (or pollen) with a chemical mutagen, and then advancing plants to a generation where mutations will be stably inherited. DNA is extracted, and seeds are stored from all members of the population to create a resource that can be accessed repeatedly over time.

For a TILLING assay, PCR primers are designed to specifically amplify a single gene target of interest. Specificity is especially important if a target is a member of a gene family or part of a polyploid genome. Next, dye-labeled primers can be used to amplify PCR products from pooled DNA of multiple individuals. These PCR products are denatured and reannealed to allow the formation of mismatched base pairs. Mismatches, or heteroduplexes, represent both naturally occurring single nucleotide polymorphisms (SNPs) (i.e., several plants from the population are likely to carry the same polymorphism) and induced SNPs (i.e., only rare individual plants are likely to display the mutation). After heteroduplex formation, the use of an endonuclease, such as Cel I, that recognizes and cleaves mismatched DNA is the key to discovering novel SNPs within a TILLING population.

Using this approach, many thousands of plants can be screened to identify any individual with a single base change as well as small insertions or deletions (1-30 bp) in any gene or specific region of the genome. Genomic fragments being assayed can range in size anywhere from 0.3 to 1.6 kb. At 8-fold pooling, 1.4 kb fragments (discounting the ends of fragments where SNP detection is problematic due to noise) and 96 lanes per assay, this combination allows up to a million base pairs of genomic DNA to be screened per single assay, making TILLING a high-throughput technique.

TILLING is further described in Slade and Knauf (2005), and Henikoff et al. (2004).

In addition to allowing efficient detection of mutations, high-throughput TILLING technology is ideal for the detection of natural polymorphisms. Therefore, interrogating an unknown homologous DNA by heteroduplexing to a known sequence reveals the number and position of polymorphic sites. Both nucleotide changes and small insertions and deletions are identified, including at least some repeat number polymorphisms. This has been called Ecotilling (Comai et al., 2004).

Each SNP is recorded by its approximate position within a few nucleotides. Thus, each haplotype can be archived based on its mobility. Sequence data can be obtained with a relatively small incremental effort using aliquots of the same amplified DNA that is used for the mismatch-cleavage assay. The left or right sequencing primer for a single reaction is chosen by its proximity to the polymorphism. Sequencher software performs a multiple alignment and discovers the base change, which in each case confirmed the gel band.

Ecotilling can be performed more cheaply than full sequencing, the method currently used for most SNP discovery. Plates containing arrayed ecotypic DNA can be screened rather than pools of DNA from mutagenized plants. Because detection is on gels with nearly base pair resolution and background patterns are uniform across lanes, bands that are of identical size can be matched, thus discovering and genotyping SNPs in a single step. In this way, ultimate sequencing of the SNP is simple and efficient, made more so by the fact that the aliquots of the same PCR products used for screening can be subjected to DNA sequencing.

Antibodies

The term "antibody" as used in this invention includes polyclonal antibodies, monoclonal antibodies, bispecific antibodies, diabodies, triabodies, heteroconjugate antibodies, chimeric antibodies including intact molecules as well as fragments thereof, such as Fab, F(ab')2, and Fv which are capable of binding the epitopic determinant, and other antibody-like molecules.

The term "specifically binds" refers to the ability of the antibody to bind to at least one polypeptide of the present invention but not significantly to known proteins in the sample/organism to be tested.

As used herein, the term "epitope" refers to a region of a polypeptide of the invention which is bound by the antibody. An epitope can be administered to an animal to generate antibodies against the epitope, however, antibodies of the present invention preferably specifically bind the epitope region in the context of the entire polypeptide.

If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is immunised with an immunogenic polypeptide of the invention. Serum from the immunised animal is collected and treated according to known procedures. If serum containing polyclonal antibodies contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art. In order that such antibodies may be made, the invention also provides polypeptides of the invention or fragments thereof haptenised to another polypeptide for use as immunogens in animals.

Monoclonal antibodies directed against polypeptides of the invention can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. Panels of monoclonal antibodies produced can be screened for various properties; i.e., for isotype and epitope affinity.

An alternative technique involves screening phage display libraries where, for example the phage express scFv fragments on the surface of their coat with a large variety of complementarity determining regions (CDRs). This technique is well known in the art.

Other techniques for producing antibodies of the invention are known in the art.

Antibodies of the invention may be bound to a solid support and/or packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like.

In an embodiment, antibodies of the present invention are detectably labeled. Exemplary detectable labels that allow for

direct measurement of antibody binding include radiolabels, fluorophores, dyes, magnetic beads, chemiluminescers, colloidal particles, and the like. Examples of labels which permit indirect measurement of binding include enzymes where the substrate may provide for a coloured or fluorescent product. Additional exemplary detectable labels include covalently bound enzymes capable of providing a detectable product signal after addition of suitable substrate. Examples of suitable enzymes for use in conjugates include horseradish peroxidase, alkaline phosphatase, malate dehydrogenase and the like. Where not commercially available, such antibody-enzyme conjugates are readily produced by techniques known to those skilled in the art. Further, exemplary detectable labels include biotin, which binds with high affinity to avidin or streptavidin; fluorochromes (e.g., phycobiliproteins, phycoerythrin and allophycocyanins; fluorescein and Texas red), which can be used with a fluorescence activated cell sorter; haptens; and the like. Preferably, the detectable label allows for direct measurement in a plate luminometer, for example, biotin. Such labeled antibodies can be used in techniques known in the art to detect polypeptides of the invention.

EXAMPLES

Example 1

Materials and Methods

Microscopic Analysis of Seedling Rust Infection

Plants were grown in a growth chamber maintained at 4-8° C. under a 12 hour light and dark regime. Seedlings were inoculated at the two leaf stage using leaf rust culture 467 and transferred into a humidity chamber (with a temperature range of 16-20° C.) for 24 hours and returned to the 4-8° C. growth chamber. For microscopic visualisation of internal infection structures, the first leaf tissue was autoclaved in 1M KOH, washed in 50 mM KPO_4 and stained with a 50 mM KPO_4 (pH 7.8) solution containing 20 ug/ml of wheat germ agglutinin (WGA) conjugated to the fluorophore alexa 488 (Invitrogen, USA) staining solution. All WGA-alexa stained tissue was examined under blue light excitation.

RNA Isolation for Semi-Quantitative PCR and Northern Blot

Total RNA was extracted from leaves using a TRIzol solution (38% Phenol, 0.8M guanidine thiocyanate, 0.4M ammonium thiocyanate, 0.1M sodium acetate pH 5 and 10% glycerol). First-strand cDNA for RT-PCR was synthesized using Superscript II reverse transcriptase (Invitrogen). A specific fragment for semi-quantitative RT-PCR of the 5' end of the PDR was amplified using the primers Lr34_RT_fl: 5'-catcaagatttcaccgcctgtgc-3' (SEQ ID NO:12) and Lr34_RT_rl: 5'-gaagcctagcaacttcacgaggc-3' (SEQ ID NO:13) at an annealing temperature of 70° C.

For the Northern blot hybridization analysis, 15 μg of total RNA per sample was blotted on a membrane (Hybond-XL, Amersham Biosiences). The probe HvS40 (Spielmeyer et al., 2002) was ^{32}P-labeled at 65° C. using the NEBlot® kit (New England BioLabs). Membranes were washed with a 0.5× SSC, 0.1% SDS solution at 65° C. and exposed to hypersensitive X-ray films (BioMax MS film, Kodak).

Rapid Amplification of cDNA Ends (RACE)

To determine the exact start of the cDNA, a 5' RACE approach was used. Poly A^+ RNA was purified from 300 μg of total RNA using the Oligotex® mRNA Mini Kit (Qiagen). Reverse transcription was done using the SMART™ RACE cDNA Amplification Kit (Clonetech Laboratories), where an adapter was ligated to the 5' end of the cDNA. Amplification of the 5' end was done using an adapter specific primer and the gene specific primer ABC_5RACE_r2: 5'-gcggggcccacaatcatctcggc-3' (SEQ ID NO:14).

Example 2

Genetic Mapping of Lr34

Plant Materials

Three backcross populations were produced and used for genetic mapping of Lr34. The parental parents for the backcrossing, scored phenotypes, population size, and markers mapped on each population are summarized in Table 2.

TABLE 2

The three backcross populations that have been used for the high-resolution genetic mapping of Lr34.

+Lr34 parent	−Lr34 parent	+Lr34 backcross line	origin	phenotypic scoring	nr. of plants	markers mapped
Forno	Arina	Arina Lr34 (Arina*3/Forno)	Swiss winter wheat	Leaf tip necrosis, leaf rust infection	1728	BE493812, SWSNP1, SWSNP2, SWSNP3, SWDEL1, SWDEL2, SWDEL3, SWM10, csLVMS
PI58548	Thatcher	RL6058 (Thatcher*6/PI58548)	Chinese landrace	Leaf, stripe, and stem rust; powdery mildew	1152	Gwm1220, BJ280740, csLVD13, csLVD2, csLVMS, BF473324, csLV34
Parula	Avocet	Avocet Lr34 (Avocet*5/Parula)	CIMMYT	Leaf and stripe rust, leaf tip necrosis	1152	Gwm1220, csLVD13, csLVD2, csLVE17, csLVMS, csLV34

The 'Arina×Formo' fine-mapping population was developed by crossing the highly resistant Swiss winter wheat cultivar 'Formo' with the susceptible Swiss winter wheat cultivar 'Arina'. Subsequent backcrossing to Arina and several generations produced by self-fertilization resulted in 103 plants that were "backcross two F4" (BC2F4) containing Lr34 and on average 12.5% of the 'Formo' genome in an otherwise Arina genetic background. These plants were analyzed for the presence of the Lr34 chromosomal segment from 'Formo' using two flanking RFLP markers BE493812 and BF473324. One of these plants containing the Lr34 region was again crossed to 'Arina' and the progeny selfed to produce 1728 BC3F2 plants, having on average 6.25% of the 'Formo' genome. Recombinants were selected using the two flanking markers BE493812 and SWM10. Phenotyping of the 'Arina×Formo' population was done at Agroscope Reckenholz, Zurich, Switzerland during 2006 (BC3F3) and 2007

(BC3F4). Infection rows containing a mixture of susceptible varieties were inoculated with urediniospores of the Swiss leaf rust isolates Nr. 90035, 91047, 93003, 93012, 94015, 95001, 95012, 95028, 95037, 95039, 95219, 95251, 96002, 96004, 96209, and 96257. Disease rating was done on two replicas.

The Thatcher×RL6058 (Thatcher Lr34) and Avocet×Avocet Lr34 high resolution mapping family, disease evaluation in Australia and at CIMMYT, Mexico were as described in Lagudah et al. (2006) and Spielmeyer et al. (2002). Other genetic stocks used in this work were the near isogenic lines 'Thatcher', 'Thatcher Lr34' (=RL6058, Thatcher*6/PI5848), 'Arina', 'Arina Lr34' (Arina*3/Formo).

Marker Development for Genetic Mapping

New molecular markers for mapping were generated by exploiting the syntenic information of rice, the model grass *Brachypodium sylvaticum* and the diploid D-genome progenitor *Aegilops tauschii* as described by Bossolini et al. (2006).

To gain physical information of the Lr34 target interval, a partially fingerprinted Bacterial Artificial Chromosome (BAC) library of *Aegilops tauschii* (J. Dvorak, U C Davis) was screened using wheat ESTs related to genes from the syntenic region of rice and *Brachypodium sylvaticum*. Thirteen BAC clones from three different contigs (HI057C6/HD036L7/HD102K14/HI056G21/HD062G18/HI031F14/HI135B2/RI004I15/RI042I4/HI148C23/BB045B13/HB067N4/BB062G18) were sequenced by low-pass sequencing using an ABI® 3730 sequencer (Applied Biosystems). Sequences were assembled using PHRAP and mined for simple sequence repeats (SSR). SSRs were amplified by designing primers in the flanking regions (Table 3).

PCR products were analyzed using the LiCOR® DNA Sequencer 4200. Polymorphic SSRs were identified and designated with prefixes "SWM" or "cs". Sequence tagged sites were developed by designing primers on low-copy sequences. Locus-specific probes were sequenced and mined for single nucleotide polymorphisms (SNP) and insertion/deletions (InDel). Polymorphic SNP-based makers and InDels were designated as Swiss Wheat SNP (SWSNP) and Swiss Wheat Deletion (SWDEL), respectively. Primer sequences for the PCR based markers mapped on the populations are summarized in Table 3. Additional low copy probes, csLVD2, csLVD13, csLVE17, for RFLP analysis were isolated from shotgun plasmid libraries from the *Ae tauschii* BAC contigs by screening with total genomic DNA from *Ae tauschii*. Recombinant plasmids where no DNA hybridization signals were detected after an overnight exposure were selected as potential low copy probes.

Using these genetic markers and the mapping populations for Lr34, the high-resolution mapping revealed a 0.15 cM target interval for Lr34 flanked by genetic markers XSWSNP3 and XcsLVE17 (FIG. **1**). Several markers (FIG. **1**) were cosegregating with Lr34.

Example 3

Mutagenesis and Isolation of Lr34 Mutants

Seeds of the Lr34 isoline, 'Lalbahadur Lr34' were irradiated using a ^{60}Co source at a dosage of 20 krad and the subsequent M1-M5 generations evaluated at CIMMYT, Mexico and in Australia as reported in Spielmeyer et al. (2002). Eight mutants were identified from the gamma-irradiated population. These were analysed using some of the new genetic markers (Example 2). Of the eight mutants, six were interstitial deletions spanning the Lr34/Yr18/Pm38/Ltn1 locus while the two mutants designated m19 and m21 showed no loss of markers in the aforementioned genetic locus. Mutants m19 and m21 were therefore subjected to further analysis utilising the newly identified markers and cosegregating genes.

Sodium azide mutants were developed using seed from a single head of an RL6058 plant grown in the glasshouse to multiply pure seed stocks for mutagenesis. Seeds were presoaked for 12 hrs at 4° C. before treating the grains in an oxygenated solution of 7 mM sodium azide at pH 3.0 for 2 hrs. The grains were rinsed and planted in the field. The M2 progenies were planted as single ear rows and scored for stripe, leaf and stem rust infection in the field in the presence of the pathogens.

TABLE 3

Primer sequences of molecular markers used in this study.

marker name	primer forward	primer reverse	marker type	Tm [° C.]
SWSNP1	5'-catctttcgtatacatga gaaac-3' (SEQ ID NO: 15)	5'-gtgtcgattcatgtgag atgc-3' (SEQ ID NO: 16)	SNP c -> t	60
SWSNP2	5'-cattatgttagcagct tagcg-3' (SEQ ID NO: 17)	5'-ccaaccatcattttggag catg-3' (SEQ ID NO: 18)	SNP c -> t	60
SWSNP3	5'-gta gat cgt gtc gtg ttc aac-3' (SEQ ID NO: 19)	5'-ctg cta atc cta agt aac gct c-3' (SEQ ID NO: 20)	SNP t -> a	65
SWDEL1	5'-cgt gag caa gac atg ggc g-3' (SEQ ID NO: 21)	5'-gct aca gct ctg aaa cta cac-3' (SEQ ID NO: 22)	6 bp InDel	66.2
SWDEL2	5'gat ttg cac gtt gat gaa acc ag-3' (SEQ ID NO: 23)	5'-cag aat gaa gtt taa cct ggc ctg-3' (SEQ ID NO: 24)	1 bp InDel	60
SWDEL3	5'- ggc tgg cta cta cga cga cg-3' (SEQ ID NO: 25)	5'-atg gtc ttt ttt cct tca gcc-3' (SEQ ID NO: 26)	180 bp InDel	65

TABLE 3-continued

Primer sequences of molecular markers used in this study.

marker name	primer forward	primer reverse	marker type	Tm [° C.]
SWM10	5′-gcc tac ttt gac ggc ata tgg-3′ (SEQ ID NO: 27)	5′-cca tct tga cat act ttg gcc ttc c-3′ (SEQ ID NO: 28)	SSR (ca)25	60
csLVMS	5′-ctc cct ccc gtg agt ata ttc-3′ (SEQ ID NO: 29)	5′-atc aaa atc cca ttg cct gac-3′ (SEQ ID NO: 30)	SSR (at)6tt(at)6	62
csLV34	5′-gtt ggt taa gac tgg tga tgg-3′ (SEQ ID NO: 31)	5′-tgc ttg cta ttg ctg aat agt-3′ (SEQ ID NO: 32)	STS	60
SWSNP1_f	5′-cat ctt tcg tat aca tga gaa ac-3′ (SEQ ID NO: 33)	5′-gtg tcg att cat gtg aga tgc-3′ (SEQ ID NO: 34)	SNP c -> t	60
SWSNP2_f	5′-cat tat gtt agc agc tta gcg-3′ (SEQ ID NO: 35)	5′-cca acc atc att ttg gag cat g-3′ (SEQ ID NO: 36)	SNP c -> t	60
SWSNP3_f	5′-gta gat cgt gtc gtg ttc aac-3′ (SEQ ID NO: 37)	5′-ctg cta atc cta agt aac gct c-3′ (SEQ ID NO: 38)	SNP t -> a	65
SWDEL1_f	5′-cgt gag caa gac atg ggc g-3′ (SEQ ID NO: 39)	5′-gct aca gct ctg aaa cta cac-3′ (SEQ ID NO: 40)	6 bp InDel	66.2
SWDEL2_f	5′gat ttg cac gtt gat gaa acc ag-3′ (SEQ ID NO: 41)	5′-cag aat gaa gtt taa cct ggc ctg-3′ (SEQ ID NO: 42)	1 bp InDel	60
SWDEL3_f	5′- ggc tgg cta cta cga cga cg-3′ (SEQ ID NO: 43)	5′-atg gtc ttt ttt cct tca gcc-3′ (SEQ ID NO: 44)	180 bp InDel	65
SWM10f	5′-gcc tac ttt gac ggc ata tgg-3′ (SEQ ID NO: 45)	5′-cca tct tga cat act ttg gcc ttc c-3′ (SEQ ID NO: 46)	SSR (ca)25	60

Six susceptible mutants were isolated and rated 70MS to 90MS for stripe rust, 50MS to 80MS for leaf rust and 50MS for stem rust under field conditions. Two mutants 4C (glycine to glutamic acid at amino acid position 1030 of SEQ ID NO:1) and 2G (glycine to aspartic acid at amino acid position 889 of SEQ ID NO:1) were the result of single nucleotide transitions that resulted in a single amino acid change within the second predicted nucleotide binding domain (FIGS. **3** and **5**). These mutants showed only partial loss of resistance to leaf rust when examined microscopically (Example 1). Mutant 2B incorporated a single nucleotide transition in exon 11 (FIG. **3**) that resulted in an early stop codon. Three mutants 3E, 4E and 2F were the result of single nucleotide transitions at splice junctions resulting in mis-spliced transcripts. The retention of introns in mutants 3E and 4E introduced early stop codons near the 5' end which was predicted to result in a non-functional protein. At the microscopic level mutants 3E and 4E were fully susceptible to leaf rust and indistinguishable from the susceptible near-isogenic line 'Thatcher'. The transcript of mutant 2F lost the second last exon (FIG. **3**) which was predicted to delete 85 amino acids from the second transmembrane domain. The 2F mutant was more susceptible to leaf rust than the susceptible control 'Thatcher' during the early infection process.

The lack of resistance resulting from loss of a functional Lr34 protein observed in the mutation study is consistent with analysis of the Lr34 gene from Jagger. Jagger has Lr34-associated alleles of the csLV34 marker but is susceptible to leaf rust and stripe rust. Sequencing of the Lr34 gene in Jagger identified a G/T point mutation that resulted in a premature stop codon. Consequently, the predicted protein of cultivar Jagger lacks 185 amino acids of the C-terminus and this allele is most likely not functional. This point mutation probably occurred in a resistant cultivar that carried the +Lr34 allele.

Example 4

Physical Information of the Target Interval and Identification of the Lr34 Gene

Two BAC libraries of the +Lr34 (resistant) cultivar 'Chinese Spring' and the –Lr34 (susceptible) cultivar 'Renan' (INRA, Toulouse, France) were screened using PCR probes covering the target interval between the two flanking markers SWSNP3 and csLVE17. The 420 kb physical interval containing both flanking markers was fully sequenced in the resistant hexaploid wheat cultivar 'Chinese Spring'. To do this, four 'Chinese Spring' BAC clones, namely 345C22, 93N17, 1964C18 and 413N16, and the 'Renan' clone 656106 were selected and fully sequenced at the Genome Sequencing Center, St. Louis, Mo., USA.

Sequence analysis revealed the presence of a gene-rich island containing ten open reading frames (FIG. **2**) encoding

proteins with homologies to two glycosyl transferases, two cysteine proteinases, two receptor lectin kinases, two cytochrome P450 proteins, a hexose carrier and an ATP binding cassette (ABC) transporter. None of these genes was present in the syntenic region in *Brachypodium sylvaticum* and only the hexose carrier was found to be conserved in the homologous region on rice chromosome 6 (rice gene Os06g0141000). Significantly and surprisingly, none of the genes appeared to be typical LRR-NBS type genes of the class commonly associated with pathogen resistance in plants. Therefore, none of the coding regions was an obvious candidate for encoding Lr34.

To determine whether one of these candidate genes corresponded to Lr34, locus-specific PCR-amplified regions corresponding to the ten candidate genes on each of the eight Lr34 mutants were sequenced. Candidate genes were amplified by developing locus-specific PCR probes, amplified from resistant and susceptible cultivars as well as on the eight Lr34 mutants, and sequenced. The mutants were the six azide mutants in the genetic background of 'Thatcher Lr34' and two gamma irradiation mutants in the 'Lalbahadur Lr34' background (Example 3).

All of the mutant lines showed a sequence alteration in the open reading frame encoding the ABC transporter (FIG. **3**). The three azide mutants 2F, 3E and 4E all had a G to A transition at an intron-exon boundary leading to splice site mutations (FIG. **7**, showing retained introns). Transitions in the two azide induced mutants 2G and 4C resulted in amino acid substitutions and line 2B carried a premature stop codon in exon 11. The two gamma irradiation mutants m19 and m21 each showed a 1 bp deletion in exon 10 and 23, respectively, leading to frame shifts and premature stop codons (FIG. **3**).

To remove the possibility of additional mutation sites in the other cosegregating genes, DNA fragments covering 12.7 kb of the other nine candidate genes and intergenic regions on the four azide mutants 2B, 3E, 4C and 4E were sequenced, without finding any additional sequence alterations. Similarly, sequencing showed that the gamma-radiation generated mutants m19 and m21 did not harbour any sequence changes in the coding regions of the remaining nine candidate genes. Therefore, the possibility that the eight mutations found in the ABC transporter were due to a very high mutation frequency in these lines could be excluded, and we concluded that the ABC transporter was responsible for conferring the durable Lr34 disease resistance.

Lr34 co-segregated with partial resistance to adult plant stripe rust (Yr18), powdery mildew (Pm38) as well as leaf tip necrosis (Ltn1). All of the mutants were more susceptible, as adult plants, to stripe rust and powdery mildew attributed to the loss of Yr18 and Pm38 and also exhibited complete or partial loss of Ltn1. These observations represented an important finding, in that eight independent mutations within a single ABC transporter gene encoding the Lr34 resistance also accounted for Yr18/Pm38/Ltn1, and demonstrated that a single gene conferred resistance to multiple pathogens.

The protein coding sequence of Lr34 spanned 11.7 kb in the wheat genome. Sequencing of the entire cDNA and comparison of the nucleotide sequence with the genomic sequence (SEQ ID NO:3) revealed that Lr34 had 24 exons. The gene contained 23 introns including a large intron of 2.5 kb between exons 4 and 5 (FIG. **3**). The protein encoded by Lr34 from the resistant cultivar Chinese Spring had 1401 amino acids (SEQ ID NO:1), while the protein from the susceptible cultivar Renan had 1402 amino acids (SEQ ID NO:4, FIG. **4**). Comparison of the amino acid sequence with other ABC transporters showed that the Lr34 proteins belonged to the Pleiotropic Drug Resistance (PDR) subfamily of ABC transporters. PDRs share a common basic structure containing two distinct domains: a cytosolic nucleotide binding domain (NBD) that contains the conserved motifs "Walker A" and "Walker B" involved in ATP binding and hydrolysis, and a hydrophobic transmembrane domain (TMD) involved in translocating the substrate. Both domains are present in duplicate, therefore the structure of PDRs is designated $[NBD\text{-}TMD]_2$ (FIG. **5**).

The PDR family is only found in fungi and plants. Fifteen PDR-like genes have been identified in the genome of *Arabidopsis* and 23 members were described in rice (Crouzet et al., 2006). It is known that PDRs confer resistance to various drugs, but little is known about the substrate specificity of this protein class (Rogers et al., 2001). It has previously been reported that PEN3/PDR8, a PDR from *Arabidopsis*, contributes to nonhost resistance to pathogens (Stein et al., 2006). The closest Lr34 homolog in rice is PDR23, showing 88% identity on the amino acid level (Table 4). In *Arabidopsis*, *Lr*34 shows closest homology to the two transporters PDR5 and PDR9, with 56% identity. The alignment of these amino acid sequences is shown in FIG. **6**.

TABLE 4

Percentage amino acid identity of wheat Lr34 to homologs of Lr34 from other plant species.

SPECIES	GenBank Accession No.	% Identity
Rice	EAZ20654	78
	EAY83289	76
	CAD59575	55
Tobacco	CAH39853 (NtPDR3)	56
Grape	CAN65735	56
Arabidopsis	NP_181265 (PDR5)	56
	NP_190916 (PDR9)	55
	DAA00881 (PDR13)	54
	DAA00869 (PDR2)	52
	NP_176196 (PDR8/PEN3)	50

The present inventors next determined the sequence differences between the Lr34 alleles in cultivars with or without Lr34-based resistance. Comparison of genomic sequences of the PDR in the +Lr34 cultivar 'Chinese Spring' and the –Lr34 French winter wheat cultivar 'Renan' revealed that the gene was present in both wheat varieties. There were only three polymorphisms in the coding sequences between these two lines (FIG. **3**). One SNP was located in the large intron 4. The other two sequence alterations were located in exons 11 and 12. A deletion of three base pairs 'TTC' found in exon 11 in 'Chinese Spring' results in the deletion of a phenylalanine residue whereas a second SNP in exon 12 converted a tyrosine to a histidine in the resistant cultivar. Both sequence differences located in exons affect the first transmembrane domain connecting the two nucleotide binding domains and they may alter the structure and binding specificity of the transporter (FIG. **4**). Sequence comparison of 2 kb of the putative promoter regions did not reveal any differences between the resistant and susceptible alleles.

To find out which of these three sequence differences were required for determining the resistance, their diagnostic value was determined on a set of +/–Lr34 genotypes sourced from different Lr34 breeding lineages (Table 5). All the +Lr34 lines showed the same haplotype as 'Chinese Spring' and all the –Lr34 lines were identical to that of 'Renan'. Hence, all of the three reported sequence differences may be important for determining the resistance conferred by Lr34, although we have no evidence that the SNP in intron 4 affects the splice efficiency of either of the alleles. Given that the same haplo-

type was found in the Lr34 PDR-ABC transporter gene for the spring wheats from the South/North American breeding programs, winter wheats from Europe and the oriental Lr34 genotypes (Table 5), we infer that a single event likely accounts for the origin of Lr34 in a wheat landrace. Evidence linking the American and European wheats containing Lr34 is traced back to the founder sib cultivars, 'Mentana' and 'Ardito' developed at the beginning of the last century (Kolmer et al., 2008).

When testing the diagnostic potential of the SNP located in intron 4 a third allele was identified. The winter wheat cultivars Zinal, Allalin and Galaxie, as well as the spelt (*Triticum spelta*) varieties Ostro and Rouquin showed the +Lr34 haplotype in intron 4, but had the –Lr34 haplotype for the two markers in exons 11 and 12. Hence, these lines form a third haplotype. Interestingly, the reciprocal allele (T, for SNP in intron 4 and +Lr34 for both exon markers) was never observed. This finding suggests that this haplotype arose through mutation rather than recombination and probably represents the progenitor of the functional +Lr34 haplotype.

TABLE 5

Polymorphisms in Lr34 alleles of wheat genotypes.

Genotype	Origin	+/– Lr34	A/T SNP	TTC/DEL	C/T SNP
Chinese Spring	China	+	A	DEL	C
RL6058*	China	+	A	DEL	C
Fukuho	Japan	+	A	DEL	C
Mentana	Italy	+		DEL	C
Frontana	Brazil	+	A	DEL	C
Frontierra	Brazil	–	T	TTC	T
Ardito	Italy	+	A	DEL	C
JupatecoR	CIMMYT	+	A	DEL	C
JupatecoS	CIMMYT	–	T	TTC	T
Glenlea	Canada	+	A	DEL	C
Thatcher	Canada	–	T	TTC	T
Anza	USA	+	A	DEL	C
Chris	USA	+	A	DEL	C
Condor	Australia	+	A	DEL	C
Penjamo 62	CIMMYT	+			
Inia66	CIMMYT	–			
LalbahadurLr34	CIMMYT	+	A	DEL	C
Lalbahadur	India	–	T	TTC	T
Forno	Switzerland	+			
Arina	Switzerland	–			
Pegaso	Italy	+	A	DEL	C
Bezostaja	Russia	+	A	DEL	C
Kavkaz	Russia	+	A	DEL	C
Roazon	France	–			
Capelle Desprez	UK	–	T	TTC	T
Maris Huntsman	UK	–	T	TTC	T
Renan	France	–	T	TTC	T
"Synthetic"_taus		–	T	TTC	T
AL8/78_taus	Armenia	–	T	TTC	T
AUS18913_taus	Iran	–	T	TTC	T

Example 5

Expression of Lr34

Lr34 is a model for adult plant resistance, which is not effective at the seedling stage under normal field conditions. To determine whether this was related to lower expression of Lr34 at the seedling stage, semi-quantitative RT-PCR was used to measure expression levels at various stages of plant development using the near isogenic lines 'Thatcher' and 'Thatcher Lr34'. The PDR was expressed at very low levels in 14 days old seedlings grown at 20° C. whereas the expression level was significantly higher in flag leaves of adult plants after 53 and 63 days (FIG. **7**). There was no substantial difference in expression between resistant and susceptible plants which was in agreement with the finding that there were no sequence differences in the promoter regions of the resistant and susceptible alleles. Interestingly, the unspliced product was observed to accumulate in adult plants after 63 days. Also, an altered transcript in 'ThatcherLr34' had 92 nucleotides missing from exon 10 which was predicted to disrupt the reading frame and result in a truncated protein.

It has been shown that Lr34 confers resistance at the seedling stage to leaf rust cultures at low temperatures (Dyck and Samborski, 1982). Analysis of the mutants and the parental Lr34 lines grown, as seedlings, at low temperatures (4-8° C.) and infected with leaf rust revealed a "slow rusting" resistance response with the intact Lr34 gene. In the initial 2-3 weeks post infection, differences in colonized mesophyl cells between mutants m19, m21 and lalbahadur Lr34' were insignificant. However by the fifth week the colonized area had extended at least four times in size with mutants m19 and m21 when compared with the active Lr34 gene. External symptoms of sporulation in seedlings were evident in the mutants by the fifth week whereas the presence of the active Lr34 gene delayed visible symptoms until after the sixth week post infection. This observation was akin to the longer latency period that was characteristic of the slow rusting mechanism of Lr34 resistance.

Lr34 conferred a broad spectrum resistance against several obligate biotrophic pathogens including fungi from the Ascomycetes and Basidiomycetes. Rubiales and Nicks (1995) reported that Lr34 was associated with reduced intercellular hyphal growth but not with a hypersensitive response or papilla formation. The eight Lr34 mutants were affected in their resistance against leaf rust, stripe rust and powdery mildew and they did not show leaf tip necrosis as described above. Infection experiments revealed that the level of resistance was coupled to the development of leaf tip necrosis and that artificial inoculation with leaf rust before emergence of leaf tip necrosis led to more severe disease symptoms than infections at later time points. These observations suggested that the resistance mechanism of Lr34 was due to a general physiological effect rather than to a 'classical' resistance mechanisms involving recognition of pathogen elicitors or secretion of antifungal components.

From this, a hypothesis was formed that the durable resistance conferred by Lr34 was associated with and at least partly due to premature senescence of the flag leaf, in particular the leaf tips. In contrast to necrosis, senescence is a highly controlled process including the remobilization of nutrients and the degradation of chlorophyll. It was considered that premature leaf senescence starting from the leaf tip could hamper the feeding of the pathogen from host cells and might retard its growth and multiplication. Senescence-related genes were therefore analysed in the wheat plants with or without Lr34.

The gene HvS40 was known to be highly upregulated during senescence in barley (Krupinska et al., 2007). A probe corresponding to this gene was prepared from cDNA. Using this probe in a Northern blot hybridization analysis revealed that wheat HvS40 was highly expressed in flag leaf tips of 'Thatcher Lr34' but not of 'Thatcher' in 63 days old plants. Furthermore the gene was down-regulated or not expressed in the six Lr34 azide mutants (FIG. **8**). This was strong evidence that Lr34 regulated senescence of flag leaves in adult wheat plants. On the other hand, microscopic observations have indicated the build up of cell wall appositions following leaf

rust infection of Lr34 genotypes. It is therefore likely that Lr34 mediated resistance affected pathogen development in a more complex way.

The cloning of Lr34 is the first reported cloning of a multi-pathogen resistance QTL from wheat, which includes Lr34, Yr18, Pm38, Ltn1 and demonstrated this was controlled by a single gene. An ABC transporter of the PDR subfamily was identified as the gene being responsible for conferring this durable adult plant resistance. Resistant and susceptible alleles differed by only three minor sequence alterations within the coding sequence. The resistant allele was thought to accelerate senescence of flag leaf tips and therefore compromise nutrient uptake by obligate biotrophic pathogens.

Example 6

Related Genes from Wheat and Other Species

The homoeologous genes from the A and B genomes of wheat, and genes encoding homologs in other species were isolated by using probes derived from the wheat Lr34 gene to probe cDNA or genomic libraries. The homoeologous genes from the A and B genomes were isolated. A homologous gene was isolated from *Aegilops tauschii*, a diploid cereal (D genome) related to wheat (SEQ ID NO:6). Other related sequences were identified from EST databases, containing partial sequences (Table 6).

TABLE 6

ESTs which are homologous to Lr34. The percentage sequence identity over the matching region is shown.

EST's	Identity	Region of SEQ ID NO: 2 corresponding to the EST
Wheat		
CJ669561	99%	1496-2333
DR733734	96%	3089-3802
CJ562397	99%	3561-4206
CV773074	100%	3732-4206
Rice		
AK102367	91%	569-2775
AK103110	91%	569-2775
CB630740	91%	1280-2085
CI097424	92%	2292-2775
CI380443	93%	2425-2775
CI361087	93%	2432-2775
CI522302	90%	1904-2252
Barley		
BU991506	71%	2518-2991
Sugarcane		
CA075859	77%	3216-3883
CA267101	77%	3407-3995

A related gene member was also detected in barley when a cDNA probe derived from the 3' half of the gene was hybridised to genomic barley DNA under standard conditions.

The present inventors have also determined the homeolog of Lr34 present on chromosome 7B of wheat. The protein sequence of this homeolog is provided as SEQ ID NO:63 and the cDNA sequence as SEQ ID NO:64.

Example 7

Production of Transgenic Wheat Expressing an Exogenous Adult Plant Pathogen Resistance Gene

In order to produce transgenic wheat, the polynucleotide comprising a sequence of nucleotides as provided in SEQ ID NO:2 is sub-cloned into a pPlex vector (Schunmann et al., 2003) such that the subterranean clover stunt virus promoter is able to drive gene transcription in a wheat cell.

Transformation of wheat embryos from the cultivar Bobwhite 26 is performed according to the method of Pellegrineschi et al. (2002). To confirm that the plants that were produced contained the construct, PCR analysis is performed on genomic DNA extracted from leaves using a FastDNA® kit (BIO 101 Inc., Vista, Calif., USA) according to the suppliers instructions. The DNA is eluted into 100 µl sterile deionized water and 1 µl used in PCR.

Plants are tested for enhanced resistance to plant pathogens such as *Puccinia graminis f.* sp. *tritici* (which causes stem rust), *Puccinia striiformis* (which causes stripe rust) and/or *Puccinia recondite f.* sp. *tritici* (which causes leaf rust).

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

The present application claims priority from AU 2008904364 filed 25 Aug. 2008, the entire contents of which are incorporated herein by reference.

All publications discussed and/or referenced herein are incorporated herein in their entirety.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

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<210> SEQ ID NO 4
<211> LENGTH: 1402
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum cv Renan

<400> SEQUENCE: 4

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Phe Thr Ala Cys Ala Ser Asp Glu Arg Pro Asp Glu Ser Glu Leu Glu
20 25 30

Leu Ala Ser Arg Gln Arg Gln Asn Gly Ala Ala Asn Thr Glu His Val
35 40 45

Ser Glu Asn Met Leu Leu Asp Ser Ser Lys Leu Gly Ala Leu Lys Arg
50 55 60

Arg Glu Phe Phe Asp Asn Leu Leu Lys Asn Leu Glu Asp Asp His Leu
65 70 75 80

Arg Phe Leu Arg Gly Gln Lys Glu Arg Ile Asp Arg Val Asp Val Lys
85 90 95

Leu Pro Ala Ile Glu Val Arg Tyr Asn Asn Leu Phe Val Glu Ala Glu
100 105 110

Cys Arg Val Thr Lys Gly Asn His Leu Pro Ser Leu Trp Asn Ser Thr
115 120 125

Lys Gly Ala Phe Ser Gly Leu Val Lys Leu Leu Gly Phe Glu Thr Glu
130 135 140

Arg Ala Lys Thr Asn Val Leu Glu Asp Val Ser Gly Ile Ile Lys Pro
145 150 155 160

Cys Arg Leu Thr Leu Leu Leu Gly Pro Pro Gly Cys Gly Lys Ser Thr
165 170 175

Leu Leu Arg Ala Leu Ala Gly Lys Leu Asp Lys Ser Leu Lys Val Thr
180 185 190

Gly Asp Ile Ser Tyr Asn Gly Tyr Glu Leu His Glu Phe Val Pro Glu
195 200 205

Lys Thr Ala Val Tyr Ile Asn Gln His Asp Leu His Ile Ala Glu Met
210 215 220

Thr Val Arg Glu Thr Leu Asp Phe Ser Ala Gln Cys Gln Gly Val Gly
225 230 235 240

Arg Arg Pro Lys Ile Leu Lys Glu Val Asn Thr Arg Glu Ser Val Ala
245 250 255

Gly Ile Ile Pro Asp Ala Asp Ile Asp Leu Tyr Met Lys Val Val Ala
260 265 270

Val Glu Ala Ser Glu Arg Ser Leu Gln Thr Asp Tyr Ile Leu Lys Ile
275 280 285

Met Gly Leu Glu Ile Cys Ala Asp Thr Met Val Gly Asp Ala Met Arg
290 295 300

Arg Gly Ile Ser Gly Gly Gln Lys Lys Arg Leu Thr Thr Ala Glu Met
305 310 315 320

Ile Val Gly Pro Ala Ser Ala Tyr Phe Met Asp Glu Ile Ser Asn Gly
325 330 335

Leu Asp Ser Ser Thr Thr Phe Gln Ile Ile Asn Cys Phe Gln Gln Leu
340 345 350

Thr Asn Ile Ser Glu Tyr Thr Met Val Ile Ser Leu Leu Gln Pro Thr
355 360 365

Pro Glu Val Phe Asp Leu Phe Asp Asp Leu Ile Leu Met Ala Glu Gly
370 375 380

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Lys Ile Ile Tyr His Gly Pro Arg Asn Glu Ala Leu Asn Phe Phe Glu
385                 390                 395                 400

Glu Cys Gly Phe Ile Cys Pro Glu Arg Lys Ala Ala Ala Asp Phe Leu
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Gln Glu Ile Leu Ser Trp Lys Asp Gln Gln Gln Tyr Trp Leu Gly Pro
            420                 425                 430

His Glu Ser Tyr Arg Tyr Ile Ser Pro His Glu Leu Ser Ser Met Phe
        435                 440                 445

Arg Glu Asn His Arg Gly Arg Lys Leu His Glu Gln Ser Val Pro Pro
    450                 455                 460

Lys Ser Gln Leu Gly Lys Glu Ala Leu Ala Phe Asn Lys Tyr Ser Leu
465                 470                 475                 480

Gln Lys Leu Glu Met Phe Lys Ala Cys Gly Ala Arg Glu Ala Leu Leu
                485                 490                 495

Met Lys Arg Asn Met Phe Val Tyr Val Phe Lys Thr Gly Gln Leu Ala
            500                 505                 510

Ile Ile Ala Leu Val Thr Met Ser Val Phe Leu Arg Thr Arg Met Thr
        515                 520                 525

Ile Ser Phe Thr His Ala Asn Tyr Tyr Met Gly Ala Leu Phe Phe Ser
    530                 535                 540

Ile Phe Met Ile Met Leu Asn Gly Ile Pro Glu Met Ser Met Gln Ile
545                 550                 555                 560

Gly Arg Leu Pro Ser Phe Tyr Lys Gln Lys Ser Tyr Tyr Phe Tyr Ser
                565                 570                 575

Ser Trp Ala Tyr Ala Ile Pro Ala Ser Val Leu Lys Val Pro Ile Ser
            580                 585                 590

Ile Leu Asp Ser Leu Val Trp Ile Ser Ile Thr Tyr Tyr Gly Ile Gly
        595                 600                 605

Tyr Thr Pro Thr Val Ser Arg Phe Phe Cys Gln Phe Leu Ile Leu Cys
    610                 615                 620

Leu Leu His His Ser Val Thr Ser Gln Tyr Arg Phe Ile Ala Ser Tyr
625                 630                 635                 640

Phe Gln Thr Pro Ile Val Ser Phe Phe Tyr Leu Phe Leu Ala Leu Thr
                645                 650                 655

Val Phe Leu Thr Phe Gly Gly Phe Ile Leu Pro Lys Thr Ser Met Pro
            660                 665                 670

Gly Trp Leu Asn Trp Gly Phe Trp Ile Ser Pro Met Thr Tyr Ala Glu
        675                 680                 685

Ile Ser Ile Val Ile Asn Glu Phe Leu Ala Pro Arg Trp Gln Lys Glu
    690                 695                 700

Ser Ile Gln Asn Ile Thr Ile Gly Asn Gln Ile Leu Val Asn His Gly
705                 710                 715                 720

Leu Tyr Tyr Ser Trp His Tyr Tyr Trp Ile Ser Phe Gly Ala Leu Leu
                725                 730                 735

Gly Ser Ile Leu Leu Phe Tyr Ile Ala Phe Gly Leu Ala Leu Asp Tyr
            740                 745                 750

Arg Thr Pro Thr Glu Glu Tyr His Gly Ser Arg Pro Thr Lys Ser Leu
        755                 760                 765

Cys Gln Gln Gln Glu Lys Asp Tyr Thr Ile Gln Asn Glu Ser Asp Asp
    770                 775                 780

Gln Ser Asn Ile Ser Lys Ala Lys Val Thr Ile Pro Val Met His Leu
785                 790                 795                 800

Pro Ile Thr Phe His Asn Leu Asn Tyr Tyr Ile Asp Thr Pro Pro Glu
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               805                 810                 815

Met Leu Lys Gln Gly Tyr Pro Thr Arg Arg Leu Arg Leu Leu Asn Asn
            820                 825                 830

Ile Thr Gly Ala Leu Arg Pro Gly Val Leu Ser Ala Leu Met Gly Val
        835                 840                 845

Ser Gly Ala Gly Lys Thr Thr Leu Leu Asp Val Leu Ala Gly Arg Lys
    850                 855                 860

Thr Gly Gly Tyr Ile Glu Gly Asp Ile Arg Ile Gly Gly Tyr Pro Lys
865                 870                 875                 880

Val Gln Glu Thr Phe Val Arg Ile Leu Gly Tyr Cys Glu Gln Val Asp
               885                 890                 895

Ile His Ser Pro Gln Leu Thr Val Glu Glu Ser Val Thr Tyr Ser Ala
            900                 905                 910

Trp Leu Arg Leu Pro Ser His Val Asp Glu Gln Thr Arg Ser Lys Phe
        915                 920                 925

Val Ala Glu Val Leu Glu Thr Val Glu Leu Asp Gln Ile Lys Asp Val
    930                 935                 940

Leu Val Gly Ser Pro Gln Lys Asn Gly Leu Ser Met Glu Gln Arg Lys
945                 950                 955                 960

Arg Leu Thr Ile Ala Val Glu Leu Val Ser Asn Pro Ser Ile Ile Leu
               965                 970                 975

Met Asp Glu Pro Thr Thr Gly Leu Asp Thr Arg Ser Ala Ala Ile Val
            980                 985                 990

Ile Arg Ala Val Lys Asn Ile Cys  Glu Thr Gly Arg Thr  Val Val Cys
        995                 1000                1005

Thr Ile  His Gln Pro Ser Thr  Glu Ile Phe Glu Ala  Phe Asp Glu
    1010                1015                1020

Leu Ile  Leu Met Lys Ser Gly  Gly Lys Thr Ile Tyr  Ser Gly Pro
    1025                1030                1035

Ile Gly  Glu Arg Ser Cys Lys  Val Ile Glu Tyr Phe  Glu Lys Ile
    1040                1045                1050

Ser Gly  Val Pro Lys Ile Lys  Ser Asn Cys Asn Pro  Ala Thr Trp
    1055                1060                1065

Met Met  Asp Val Thr Ser Thr  Ser Met Glu Val Gln  His Asn Met
    1070                1075                1080

Asp Phe  Ala Ile Leu Tyr Glu  Glu Ser Ser Leu His  Arg Glu Ala
    1085                1090                1095

Glu Asp  Leu Val Glu Gln Leu  Ser Ile Pro Leu Pro  Asn Ser Glu
    1100                1105                1110

Asn Leu  Cys Phe Ser His Ser  Phe Ala Gln Asn Gly  Trp Ile Gln
    1115                1120                1125

Leu Lys  Ala Cys Leu Trp Lys  Gln Asn Ile Thr Tyr  Trp Arg Ser
    1130                1135                1140

Pro Gln  Tyr Asn Leu Arg Arg  Ile Met Met Thr Val  Ile Ser Ala
    1145                1150                1155

Leu Ile  Tyr Gly Ile Leu Phe  Trp Lys His Ala Lys  Val Leu Asn
    1160                1165                1170

Asn Glu  Gln Asp Met Leu Ser  Val Phe Gly Ala Met  Tyr Leu Gly
    1175                1180                1185

Phe Thr  Thr Ile Gly Ala Tyr  Asn Asp Gln Thr Ile  Ile Pro Phe
    1190                1195                1200

Ser Thr  Thr Glu Arg Ile Val  Met Tyr Arg Glu Arg  Phe Ala Gly
    1205                1210                1215
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Met Tyr  Ser Ser Trp Ser Tyr  Ser Phe Ala Gln Ala  Phe Ile Glu
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Ile Pro  Tyr Val Phe Ile Gln  Val Val Leu Tyr Thr  Leu Ile Val
    1235                1240                1245

Tyr Pro  Ser Thr Gly Tyr Tyr  Trp Thr Ala His Lys  Phe Leu Trp
    1250                1255                1260

Phe Phe  Tyr Thr Thr Phe Cys  Ser Ile Leu Ser Tyr  Val Tyr Val
    1265                1270                1275

Gly Leu  Leu Leu Val Ser Ile  Thr Pro Asn Val Gln  Val Ala Thr
    1280                1285                1290

Ile Leu  Ala Ser Phe Phe Asn  Thr Met Gln Thr Leu  Phe Ser Gly
    1295                1300                1305

Phe Ile  Leu Pro Ala Pro Gln  Ile Pro Lys Trp Trp  Thr Trp Leu
    1310                1315                1320

Tyr Tyr  Leu Thr Pro Thr Ser  Trp Ala Leu Asn Ala  Leu Leu Thr
    1325                1330                1335

Ser Gln  Tyr Gly Asn Ile Glu  Lys Glu Val Lys Ala  Phe Gly Glu
    1340                1345                1350

Thr Lys  Ser Val Ser Ile Phe  Leu Asn Asp Tyr Phe  Gly Phe His
    1355                1360                1365

Gln Asp  Lys Leu Ser Val Val  Ala Ala Val Leu Val  Ala Phe Pro
    1370                1375                1380

Phe Val  Leu Ile Ile Leu Phe  Ser Leu Ser Ile Glu  Lys Leu Asn
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Phe Gln  Lys Arg
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<210> SEQ ID NO 5
<211> LENGTH: 4209
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum cv Renan

<400> SEQUENCE: 5

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gctctcaaga ggcgcgagtt cttcgacaac ctgctaaaga acctcgaaga cgaccacctc    240

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atgtaagaca ttttttgacc ctttgaataa tatatctatg atataaacat tggtttgagc 10860

taagtaggat tggtatctaa attatagtaa gatgcttaac aactatttac tactccctct 10920

gtctcaaaat gtaagacatt ttttgacaca tgtaagacgt tttttgacat tatcagtgtc 10980

aaaaaatgtc ttacattttg agatagaggg agcatctagc atccttttc tgtttcggga 11040

gaacatttct taccttaaat atttggcata tacttttttgt acaaaaacaa ccccattata 11100

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aacttggatt cccgcaccgt catcatcatg ccttaatatc cgtgcaaggt gtgtgagtgg 14640

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<210> SEQ ID NO 7
<211> LENGTH: 841
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 7

ggaatatgtt tgtttatgtc ttcaaaacag gccagcttgc cattattgca ctcgtaacaa 60

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tgtctgtatt ccttcgaact cgcatgacaa taagtttcac tcatgcaaat tactatatgg 120

gagcattatt tttttccatc ttcatgatta tgttaaatgg cataccagag atgagcatgc 180

agattgggag actcccaagt ttttacaagc aaaagagcta ctatttctat tcatcatggg 240

catatgcaat accagcttca gtcctaaagg tccctatttc catactggat tcgcttgtat 300

ggatatctat cacatattat ggtattggtt atacacctac tgtttcaagg ttcttctgcc 360

agtttctgat actttgtctt ctccatcatt cagtcacctc gcagtatcga tttattgctt 420

catacttcca aacacctatt gtgtctttct tctacctttt tcttgctcta acagtattcc 480

ttacattcgg aggcttcatt cttcccaaga cctccatgcc aggatggtta aactggggat 540

tttggatatc tccaatgaca tatgcagaaa tcagcatagt tattaacgaa ttcttggcac 600

caagatggca gaaggaaagt attcaaaaca taacaattgg gaaccaaatc ctggttaatc 660

atggcctata ttacagttgg cattattatt ggatatcctt tggagccttg cttggatcta 720

ttctcttatt ttatatcgct tttggattgg cactagatta cagaacacct acagaagaat 780

atcatggaag caggcctaca aagagcttat gtcaacagca ggaaaaagat tacactattc 840

a 841

<210> SEQ ID NO 8
<211> LENGTH: 821
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 8

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gctcgtaccg gtccggaatt cccgggatat cgtcgaccca cgcgtccgga gaacaatcta 120

ccgcggacca ataagagagc gctcctgcaa agtgatccag tactttgaga gaattgcggg 180

agtcccaaaa ataaagagta actgcaatcc acctacttgg atgatggatg taacatccac 240

atcaatggag gttcaacaca atatggactt tgcaattttg tatgaagagt cgtcactgca 300

tatagaagct gaagatctag tggagcagct aagtatccca ttaccaaatt cagaaaatct 360

atgtttctcc cataatgtta cacagaatgg ctggattcaa cttaaagctt gcttgtggaa 420

acaagacata acttactgga ggagtcctca gtataacttg aggcgcatta tgatgactgt 480

catatctgcc ctgatctacg gaatattggt ctggaagcat gcaaaagtat taaacaacga 540

gcacgacatg ctcagtgttt ttggcgcaat gtatttggga ttcaccacca taggcgctta 600

taatgatcag acaatcatac cattcagtac aactgatcgt attgtaatgt atcgtgagag 660

atttgcagga atgtattcat cttggtcata ttcattcgca caggctttgg ttgacatacc 720

ctatgtattt atccaagtgg tactgtatac gttaattgtc tatcggtcaa ctggttatta 780

ttggacagca cccaaattcc tatggttctt ctacaccaca t 821

<210> SEQ ID NO 9
<211> LENGTH: 865
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 9

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ttattgtaca caattactta ttcatgaggt ttacccatca gatttgagaa aaatagccac 120

ccaatcagct ctgattctgc atttcgcagt tggaaaaaag gcaagtagct atatctgtaa 180

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catgtacaga ttcatgtctg ttggaatgtc agaacttgct tacctcttct ggaaattaag 240

tttctcaatg gacaacgaaa acaagattat caacacaaaa ggaaaggcaa cgaggacagc 300

tgctactacg ctcaacttat cttgatgaaa cccaaaatag tcattcaaga agattgaaac 360

tgatttagtt tctccaaatg ctttcacctc ttttttctatg tttccgtatt gtgatgtcaa 420

gagggcattg agtgcccaag atgtaggagt gagatagtag agccaagtcc accacttcgg 480

gatttgaggt gcaggtaaaa taaatcctga gaatagtgtt tgcatggtgt tgaaaaatga 540

agccagtatg gtagctactt gaacattggg ggttatggaa acaagaagca acccaacata 600

aacataggag agaattgaac aaaatgtggt gtagaagaac cataggaatt tgtgtgctgt 660

ccaataataa ccagttgacg gatagacaat taacgtatac agtaccactt ggataaatac 720

atagggtatc tcaatgaaag cctgtgcgaa tgaatatgac caagatgaat acattcctgc 780

aaatctctca cgatacatta caatacgctc agttgtactg aatggtatga ttgtctgatc 840

attataagcg cctatggggg tgaaa 865

<210> SEQ ID NO 10
<211> LENGTH: 810
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 10

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ctggttatta ttggacagca cacaaattcc tatggttctt ctacaccaca ttttgttcaa 180

ttctctccta tgtttatgtt gggttgcttc ttgtttccat aacccccaat gttcaagtag 240

ctaccatact ggcttcattt ttcaacacca tgcaaacact attctcagga tttattttac 300

ctgcacctca aatcccgaag tggtggactt ggctctacta tctcactcct acatcttggg 360

cactcaatgc cctcttgaca tcacaatacg gaaacataga aaaagaggtg aaagcatttg 420

gagaaactaa atcagtttca atcttcttga atgactattt tgggtttcat caagataagt 480

tgagcgtagt agcagctgtc ctcgttgcct ttccttttgt gttgataatc ttgttttcgt 540

tgtccattga gaaacttaat ttccagaaga ggtaagcaag ttctgacatt ccaacagaca 600

tgaatctgta catgttacag atatagctac ttgccttttt tcaactgcga aatgcagaat 660

cagagctgat tgggtggcta tttttctcaa atctgatggg taaacctcat gaataagtaa 720

ttgtgtacaa taacttgatt gtgctaagta cgattgtgag ttgtaatctt tttgtttcac 780

cgttcagaag aatttgatgg ttacaaatca 810

<210> SEQ ID NO 11
<211> LENGTH: 571
<212> TYPE: DNA
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 11

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acgggagcat tccagccagg ggttctctcg gcactcatgg gggttactgg agcggaaaaa 120

caacactcct tgatgttctt gctggaagga aaactggcgg tgttattgaa ggggatataa 180

gaataggagg gtatcctaaa attcagcaga cttttgctag gatatcaggc tactgtgaac 240

aaactgatgt ccattcccca caaatcacag tgggtgaatc ggttgcatat tcagcctggt 300

tacgccttcc accagaagtt gatgcaaaaa taagaaccga atttgtcaac gaagttcttg 360

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aaacaattga gttggacgaa attagagatt ctttggtcgg aatacctggg gtaaatgggc 420

tatcaacaga gcaaaggaaa cggctcacga ttgcagtcga gctcgtgtct aacccctcaa 480

tcatatttat ggacgagcca acgtcaggct tggatgcaag ggccgctgct attgtcatgc 540

gtgcagtgaa gaatgttgca gacacaggcc g 571

<210> SEQ ID NO 12
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 12

catcaagatt tcaccgcctg tgc 23

<210> SEQ ID NO 13
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 13

gaagcctagc aacttcacga ggc 23

<210> SEQ ID NO 14
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 14

gcggggccca caatcatctc ggc 23

<210> SEQ ID NO 15
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 15

catctttcgt atacatgaga aac 23

<210> SEQ ID NO 16
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 16

gtgtcgattc atgtgagatg c 21

<210> SEQ ID NO 17
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 17

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cattatgtta gcagcttagc g 21

<210> SEQ ID NO 18
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 18

ccaaccatca ttttggagca tg 22

<210> SEQ ID NO 19
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 19

gtagatcgtg tcgtgttcaa c 21

<210> SEQ ID NO 20
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 20

ctgctaatcc taagtaacgc tc 22

<210> SEQ ID NO 21
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 21

cgtgagcaag acatgggcg 19

<210> SEQ ID NO 22
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 22

gctacagctc tgaaactaca c 21

<210> SEQ ID NO 23
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 23

gatttgcacg ttgatgaaac cag 23

<210> SEQ ID NO 24
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 24

cagaatgaag tttaacctgg cctg 24

<210> SEQ ID NO 25
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 25

ggctggctac tacgacgacg 20

<210> SEQ ID NO 26
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 26

atggtctttt ttccttcagc c 21

<210> SEQ ID NO 27
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 27

gcctactttg acggcatatg g 21

<210> SEQ ID NO 28
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 28

ccatcttgac atactttggc cttcc 25

<210> SEQ ID NO 29
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 29

ctccctcccg tgagtatatt c 21

<210> SEQ ID NO 30
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 30

atcaaaatcc cattgcctga c 21

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<210> SEQ ID NO 31
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 31

gttggttaag actggtgatg g 21

<210> SEQ ID NO 32
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 32

tgcttgctat tgctgaatag t 21

<210> SEQ ID NO 33
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 33

catctttcgt atacatgaga aac 23

<210> SEQ ID NO 34
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 34

gtgtcgattc atgtgagatg c 21

<210> SEQ ID NO 35
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 35

cattatgtta gcagcttagc g 21

<210> SEQ ID NO 36
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 36

ccaaccatca ttttggagca tg 22

<210> SEQ ID NO 37
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

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<400> SEQUENCE: 37

gtagatcgtg tcgtgttcaa c 21

<210> SEQ ID NO 38
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 38

ctgctaatcc taagtaacgc tc 22

<210> SEQ ID NO 39
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 39

cgtgagcaag acatgggcg 19

<210> SEQ ID NO 40
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 40

gctacagctc tgaaactaca c 21

<210> SEQ ID NO 41
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 41

gatttgcacg ttgatgaaac cag 23

<210> SEQ ID NO 42
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 42

cagaatgaag tttaacctgg cctg 24

<210> SEQ ID NO 43
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 43

ggctggctac tacgacgacg 20

<210> SEQ ID NO 44

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<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 44

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<210> SEQ ID NO 45
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 45

gcctactttg acggcatatg g 21

<210> SEQ ID NO 46
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 46

ccatcttgac atactttggc cttcc 25

<210> SEQ ID NO 47
<211> LENGTH: 1406
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa

<400> SEQUENCE: 47

Met Ser Ser Ser Ser Ser His His Pro Glu Phe Ala Ser Cys Thr Ala
1 5 10 15

Asn Asp Asp Glu His His Leu Asp Glu Phe Glu Leu Glu Leu Val Val
20 25 30

Gln Asp Val Gln Arg Gln Gln Asn Asn Gly Ser Ala Asn Thr Asp Gln
35 40 45

His Glu Arg Glu Asn Leu Leu Leu Leu Asp Asp Ser Ser Lys Ser Gly
50 55 60

Ala Leu Lys Glu Arg Leu Phe Phe Asp Asn Leu Leu Lys Asn Val Gln
65 70 75 80

Asp Asp His Ile Arg Phe Leu His Arg Gln Lys Glu Arg Ile Asp Arg
85 90 95

Val Asp Val Lys Leu Pro Ala Ile Glu Val Arg Tyr Asn Asn Leu Ser
100 105 110

Val Glu Ala Glu Cys Arg Thr Ala Asn Gly Asp His Leu Pro Ser Leu
115 120 125

Trp Asn Ser Thr Lys Gly Ala Phe Ser Gly Leu Val Lys Leu Leu Gly
130 135 140

Leu Glu Thr Glu Arg Ala Lys Ile Asn Val Leu Glu Asp Val Ser Gly
145 150 155 160

Ile Ile Lys Pro Cys Arg Leu Thr Leu Leu Leu Gly Pro Pro Gly Cys
165 170 175

Gly Lys Ser Thr Leu Leu Arg Ala Leu Ser Gly Lys Leu Asp Lys Ser
180 185 190

Leu Lys Val Thr Gly Asp Ile Ser Tyr Asn Gly Tyr Gln Leu Asp Glu

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        195                 200                 205

Phe Val Pro Glu Lys Thr Ala Ala Tyr Ile Ser Gln Tyr Asp Leu His
    210                 215                 220

Ile Pro Glu Met Thr Val Arg Glu Thr Leu Asp Phe Ser Ser Arg Cys
225                 230                 235                 240

Gln Gly Val Gly Arg Arg Pro Lys Ile Leu Lys Glu Val Ser Ala Arg
                245                 250                 255

Glu Ser Ala Ala Gly Ile Ile Pro Asp Ala Asp Ile Asp Ile Tyr Met
            260                 265                 270

Lys Ala Ile Ser Val Glu Ala Ser Lys Arg Ser Leu Gln Thr Asp Tyr
        275                 280                 285

Ile Leu Lys Ile Met Gly Leu Glu Ile Cys Ala Asp Thr Met Val Gly
    290                 295                 300

Asp Ala Met Ile Arg Gly Leu Ser Gly Gly Gln Lys Lys Arg Leu Thr
305                 310                 315                 320

Thr Ala Glu Met Ile Val Gly Pro Ala Arg Ala Tyr Phe Met Asp Glu
                325                 330                 335

Ile Ser Asn Gly Leu Asp Ser Ser Thr Thr Phe Gln Ile Ile Ser Cys
            340                 345                 350

Phe Gln Gln Leu Thr Asn Ile Ser Glu Tyr Thr Met Val Ile Ser Leu
        355                 360                 365

Leu Gln Pro Thr Pro Glu Val Phe Asp Leu Phe Asp Asp Leu Ile Leu
    370                 375                 380

Met Ala Glu Gly Lys Ile Ile Tyr His Gly Pro Arg Asn Glu Ala Leu
385                 390                 395                 400

Asn Phe Phe Glu Glu Cys Gly Phe Ile Cys Pro Glu Arg Lys Glu Val
                405                 410                 415

Ala Asp Phe Leu Gln Glu Ile Leu Ser Cys Lys Asp Gln Gln Gln Tyr
            420                 425                 430

Trp Ser Gly Pro Asn Glu Ser Tyr Arg Tyr Ile Ser Pro His Glu Leu
        435                 440                 445

Ser Ser Met Phe Lys Glu Asn His Arg Gly Arg Lys Leu Glu Glu Pro
    450                 455                 460

Ile Val Ser Pro Lys Ser Glu Leu Gly Lys Glu Ala Leu Ala Phe Asn
465                 470                 475                 480

Lys Tyr Ser Leu Gln Lys Leu Glu Met Phe Lys Ala Cys Gly Ala Arg
                485                 490                 495

Glu Ala Leu Leu Met Lys Arg Ser Met Phe Val Tyr Val Phe Lys Thr
            500                 505                 510

Gly Gln Leu Ala Ile Ile Ala Leu Val Thr Met Ser Val Phe Leu Arg
        515                 520                 525

Thr Arg Met Thr Thr Asp Phe Thr His Ala Thr Tyr Tyr Met Gly Ala
    530                 535                 540

Leu Phe Phe Ser Ile Leu Met Ile Met Leu Asn Gly Thr Pro Glu Ile
545                 550                 555                 560

Ser Met Gln Ile Arg Arg Leu Pro Ser Phe Tyr Lys Gln Lys Ser Tyr
                565                 570                 575

Tyr Phe Tyr Ser Ser Trp Ala Tyr Ala Ile Pro Ala Ser Val Leu Lys
            580                 585                 590

Val Pro Val Ser Ile Leu Asp Ser Leu Val Trp Ile Cys Ile Thr Tyr
        595                 600                 605

Tyr Gly Ile Gly Tyr Thr Ala Ser Val Ser Arg Phe Phe Cys Gln Phe
    610                 615                 620
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Leu Met Leu Cys Phe Val His Gln Ser Val Thr Ser Leu Tyr Arg Phe
625                 630                 635                 640

Ile Ala Ser Tyr Phe Gln Thr Pro Thr Ala Ser Phe Phe Tyr Leu Phe
                645                 650                 655

Leu Ala Leu Thr Phe Phe Leu Met Phe Gly Gly Phe Thr Leu Pro Lys
            660                 665                 670

Pro Ser Met Pro Gly Trp Leu Asn Trp Gly Phe Trp Ile Ser Pro Met
        675                 680                 685

Thr Tyr Ala Glu Ile Gly Thr Val Ile Asn Glu Phe Gln Ala Pro Arg
    690                 695                 700

Trp Gln Lys Glu Thr Ile Gln Asn Ile Thr Ile Gly Asn Arg Ile Leu
705                 710                 715                 720

Ile Asn His Gly Leu Tyr Tyr Ser Trp His Phe Tyr Trp Ile Ser Ile
                725                 730                 735

Gly Ala Leu Phe Gly Ser Ile Ile Leu Phe Tyr Ile Ala Phe Gly Leu
            740                 745                 750

Ala Leu Asp Tyr Ile Thr Ser Ile Glu Glu Tyr His Gly Ser Arg Pro
        755                 760                 765

Ile Lys Arg Leu Cys Gln Glu Gln Glu Lys Asp Ser Asn Ile Arg Lys
    770                 775                 780

Glu Ser Asp Gly His Ser Asn Ile Ser Arg Ala Lys Met Thr Ile Pro
785                 790                 795                 800

Val Met Glu Leu Pro Ile Thr Phe His Asn Leu Asn Tyr Tyr Ile Asp
                805                 810                 815

Thr Pro Pro Glu Met Leu Lys Gln Gly Tyr Pro Thr Lys Arg Leu Gln
            820                 825                 830

Leu Leu Asn Asn Ile Thr Gly Ala Leu Arg Pro Gly Val Leu Ser Ala
        835                 840                 845

Leu Met Gly Val Ser Gly Ala Gly Lys Thr Thr Leu Leu Asp Val Leu
    850                 855                 860

Ala Gly Arg Lys Thr Gly Gly Tyr Ile Glu Gly Asp Ile Arg Ile Gly
865                 870                 875                 880

Gly Tyr Pro Lys Val Gln Glu Thr Phe Val Arg Ile Leu Gly Tyr Cys
                885                 890                 895

Glu Gln Ala Asp Ile His Ser Pro Gln Leu Thr Val Glu Glu Ser Val
            900                 905                 910

Thr Tyr Ser Ala Trp Leu Arg Leu Pro Ser His Val Asp Lys Lys Thr
        915                 920                 925

Arg Ser Glu Phe Val Ala Glu Val Leu Glu Thr Val Glu Leu Asp Gln
    930                 935                 940

Ile Lys Asp Val Leu Val Gly Thr Pro Gln Lys Asn Gly Leu Ser Met
945                 950                 955                 960

Glu Gln Arg Lys Arg Leu Thr Ile Ala Val Glu Leu Val Ser Asn Pro
                965                 970                 975

Ser Val Ile Leu Met Asp Glu Pro Thr Thr Gly Leu Asp Thr Arg Ser
            980                 985                 990

Ala Ala Ile Val Ile Arg Ala Val  Lys Asn Ile Cys Lys  Thr Gly Arg
        995                 1000                1005

Thr Val  Val Cys Thr Ile His  Gln Pro Ser Thr Lys  Ile Phe Glu
    1010                1015                1020

Ala Phe  Asp Glu Leu Ile Leu  Met Lys Asn Gly Gly  Lys Ile Ile
    1025                1030                1035
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Tyr Asn  Gly Pro Ile Gly Glu  Arg Ser Ser Lys Val  Ile Glu Tyr
    1040                1045                1050

Phe Glu  Lys Ile Ser Gly Val  Leu Lys Val Lys Ser  Asn Cys Asn
    1055                1060                1065

Pro Ala  Ala Trp Met Met Asp  Val Thr Ser Thr Ser  Met Glu Val
    1070                1075                1080

Gln His  Asn Met Asp Phe Ala  Ile Leu Tyr Asp Glu  Ser Ser Gln
    1085                1090                1095

His Arg  Asp Ile Val Glu Leu  Val Glu Lys Leu Ser  Ile Pro Ile
    1100                1105                1110

Pro Asn  Ser Glu Ile Leu Ser  Phe Ser His Arg Phe  Pro Arg Asn
    1115                1120                1125

Gly Trp  Ile Gln Leu Lys Ala  Cys Leu Trp Lys Gln  Asn Leu Thr
    1130                1135                1140

Tyr Trp  Arg Ser Pro Glu Tyr  Asn Leu Arg Arg Ile  Met Leu Thr
    1145                1150                1155

Val Ile  Ser Ala Leu Val Tyr  Gly Val Leu Phe Trp  Lys Arg Ala
    1160                1165                1170

Lys Ile  Leu Asn Asp Glu Gln  Asp Leu Phe Asn Val  Phe Gly Ala
    1175                1180                1185

Met Tyr  Leu Gly Ser Thr Thr  Ile Gly Ser Tyr Asn  His Gln Ser
    1190                1195                1200

Ile Ile  Pro Phe Ser Thr Thr  Glu Arg Ile Val Met  Tyr Arg Glu
    1205                1210                1215

Lys Phe  Ala Gly Met Tyr Ser  Ser Trp Ser Tyr Ser  Phe Ala Gln
    1220                1225                1230

Ala Ala  Ile Glu Ile Pro Tyr  Val Phe Ile Gln Val  Val Leu Tyr
    1235                1240                1245

Thr Leu  Ile Ile Tyr Pro Ser  Ile Gly Tyr Tyr Trp  Thr Thr His
    1250                1255                1260

Lys Phe  Ile Trp Phe Phe Tyr  Thr Thr Phe Cys Ser  Ser Leu Ser
    1265                1270                1275

Tyr Ile  Tyr Val Gly Leu Leu  Leu Val Ser Leu Thr  Pro Asn Val
    1280                1285                1290

Gln Val  Ala Thr Ile Leu Ala  Ser Phe Phe Asn Thr  Met Gln Thr
    1295                1300                1305

Leu Phe  Ser Gly Phe Ile Leu  Pro Ala Pro Gln Ile  Pro Lys Trp
    1310                1315                1320

Trp Val  Trp Leu Tyr Tyr Leu  Thr Pro Thr Ser Trp  Thr Leu Asp
    1325                1330                1335

Ala Leu  Leu Thr Ser Gln Tyr  Gly Asn Ile Glu Lys  Glu Val Arg
    1340                1345                1350

Ala Phe  Gly Glu Thr Lys Ser  Val Ser Ile Phe Leu  Asn Asp Tyr
    1355                1360                1365

Phe Gly  Phe His Lys Asp Lys  Leu Ser Leu Val Ala  Ala Val Leu
    1370                1375                1380

Ile Ala  Phe Pro Phe Val Leu  Ile Ile Leu Phe Ser  Phe Ser Ile
    1385                1390                1395

Glu Lys  Phe Asn Phe Gln Lys  Arg
    1400                1405
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<210> SEQ ID NO 48
<211> LENGTH: 1413
<212> TYPE: PRT

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<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 48

Met Gly Ser Ser Phe Arg Ser Ser Ser Ser Arg Asn Glu His Glu Asp
1 5 10 15

Gly Gly Asp Glu Ala Glu His Ala Leu Gln Trp Ala Glu Ile Gln Arg
20 25 30

Leu Pro Thr Phe Lys Arg Leu Arg Ser Ser Leu Val Asp Lys Tyr Gly
35 40 45

Glu Gly Thr Glu Lys Gly Lys Lys Val Val Asp Val Thr Lys Leu Gly
50 55 60

Ala Met Glu Arg His Leu Met Ile Glu Lys Leu Ile Lys His Ile Glu
65 70 75 80

Asn Asp Asn Leu Lys Leu Leu Lys Lys Ile Arg Arg Arg Met Glu Arg
85 90 95

Val Gly Val Glu Phe Pro Ser Ile Glu Val Arg Tyr Glu His Leu Gly
100 105 110

Val Glu Ala Ala Cys Glu Val Val Glu Gly Lys Ala Leu Pro Thr Leu
115 120 125

Trp Asn Ser Leu Lys His Val Phe Leu Asp Leu Leu Lys Leu Ser Gly
130 135 140

Val Arg Thr Asn Glu Ala Asn Ile Lys Ile Leu Thr Asp Val Ser Gly
145 150 155 160

Ile Ile Ser Pro Gly Arg Leu Thr Leu Leu Leu Gly Pro Pro Gly Cys
165 170 175

Gly Lys Thr Thr Leu Leu Lys Ala Leu Ser Gly Asn Leu Glu Asn Asn
180 185 190

Leu Lys Cys Tyr Gly Glu Ile Ser Tyr Asn Gly His Gly Leu Asn Glu
195 200 205

Val Val Pro Gln Lys Thr Ser Ala Tyr Ile Ser Gln His Asp Leu His
210 215 220

Ile Ala Glu Met Thr Thr Arg Glu Thr Ile Asp Phe Ser Ala Arg Cys
225 230 235 240

Gln Gly Val Gly Ser Arg Thr Asp Ile Met Met Glu Val Ser Lys Arg
245 250 255

Glu Lys Asp Gly Gly Ile Ile Pro Asp Pro Glu Ile Asp Ala Tyr Met
260 265 270

Lys Ala Ile Ser Val Lys Gly Leu Lys Arg Ser Leu Gln Thr Asp Tyr
275 280 285

Ile Leu Lys Ile Leu Gly Leu Asp Ile Cys Ala Glu Thr Leu Val Gly
290 295 300

Asn Ala Met Lys Arg Gly Ile Ser Gly Gly Gln Lys Lys Arg Leu Thr
305 310 315 320

Thr Ala Glu Met Ile Val Gly Pro Thr Lys Ala Leu Phe Met Asp Glu
325 330 335

Ile Thr Asn Gly Leu Asp Ser Ser Thr Ala Phe Gln Ile Ile Lys Ser
340 345 350

Leu Gln Gln Val Ala His Ile Thr Asn Ala Thr Val Phe Val Ser Leu
355 360 365

Leu Gln Pro Ala Pro Glu Ser Tyr Asp Leu Phe Asp Asp Ile Val Leu
370 375 380

Met Ala Glu Gly Lys Ile Val Tyr His Gly Pro Arg Asp Asp Val Leu
385 390 395 400

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Lys Phe Phe Glu Glu Cys Gly Phe Gln Cys Pro Glu Arg Lys Gly Val
                405                 410                 415

Ala Asp Phe Leu Gln Glu Val Ile Ser Lys Lys Asp Gln Gly Gln Tyr
            420                 425                 430

Trp Leu His Gln Asn Leu Pro His Ser Phe Val Ser Val Asp Thr Leu
        435                 440                 445

Ser Lys Arg Phe Lys Asp Leu Glu Ile Gly Arg Lys Ile Glu Glu Ala
    450                 455                 460

Leu Ser Lys Pro Tyr Asp Ile Ser Lys Thr His Lys Asp Ala Leu Ser
465                 470                 475                 480

Phe Asn Val Tyr Ser Leu Pro Lys Trp Glu Leu Phe Arg Ala Cys Ile
                485                 490                 495

Ser Arg Glu Phe Leu Leu Met Lys Arg Asn Tyr Phe Val Tyr Leu Phe
            500                 505                 510

Lys Thr Phe Gln Leu Val Leu Ala Ala Ile Ile Thr Met Thr Val Phe
        515                 520                 525

Ile Arg Thr Arg Met Asp Ile Asp Ile Ile His Gly Asn Ser Tyr Met
    530                 535                 540

Ser Cys Leu Phe Phe Ala Thr Val Val Leu Leu Val Asp Gly Ile Pro
545                 550                 555                 560

Glu Leu Ser Met Thr Val Gln Arg Leu Ser Val Phe Tyr Lys Gln Lys
                565                 570                 575

Gln Leu Cys Phe Tyr Pro Ala Trp Ala Tyr Ala Ile Pro Ala Thr Val
            580                 585                 590

Leu Lys Ile Pro Leu Ser Phe Phe Glu Ser Leu Val Trp Thr Cys Leu
        595                 600                 605

Thr Tyr Tyr Val Ile Gly Tyr Thr Pro Glu Pro Tyr Arg Phe Phe Arg
    610                 615                 620

Gln Phe Met Ile Leu Phe Ala Val His Phe Thr Ser Ile Ser Met Phe
625                 630                 635                 640

Arg Cys Ile Ala Ala Ile Phe Gln Thr Gly Val Ala Ala Met Thr Ala
                645                 650                 655

Gly Ser Phe Val Met Leu Ile Thr Phe Val Phe Ala Gly Phe Ala Ile
            660                 665                 670

Pro Tyr Thr Asp Met Pro Gly Trp Leu Lys Trp Gly Phe Trp Val Asn
        675                 680                 685

Pro Ile Ser Tyr Ala Glu Ile Gly Leu Ser Val Asn Glu Phe Leu Ala
    690                 695                 700

Pro Arg Trp Gln Lys Met Gln Pro Thr Asn Val Thr Leu Gly Arg Thr
705                 710                 715                 720

Ile Leu Glu Ser Arg Gly Leu Asn Tyr Asp Asp Tyr Met Tyr Trp Val
                725                 730                 735

Ser Leu Ser Ala Leu Leu Gly Leu Thr Ile Ile Phe Asn Thr Ile Phe
            740                 745                 750

Thr Leu Ala Leu Ser Phe Leu Lys Ser Pro Thr Ser Ser Arg Pro Met
        755                 760                 765

Ile Ser Gln Asp Lys Leu Ser Glu Leu Gln Gly Thr Lys Asp Ser Ser
    770                 775                 780

Val Lys Lys Asn Lys Pro Leu Asp Ser Ser Ile Lys Thr Asn Glu Asp
785                 790                 795                 800

Pro Gly Lys Met Ile Leu Pro Phe Lys Pro Leu Thr Ile Thr Phe Gln
                805                 810                 815

Asp Leu Asn Tyr Tyr Val Asp Val Pro Val Glu Met Lys Gly Gln Gly
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           820                 825                 830

Tyr Asn Glu Lys Lys Leu Gln Leu Leu Ser Glu Ile Thr Gly Ala Phe
        835                 840                 845

Arg Pro Gly Val Leu Thr Ala Leu Met Gly Ile Ser Gly Ala Gly Lys
    850                 855                 860

Thr Thr Leu Leu Asp Val Leu Ala Gly Arg Lys Thr Ser Gly Tyr Ile
865                 870                 875                 880

Glu Gly Glu Ile Arg Ile Ser Gly Phe Leu Lys Val Gln Glu Thr Phe
                885                 890                 895

Ala Arg Val Ser Gly Tyr Cys Glu Gln Thr Asp Ile His Ser Pro Ser
            900                 905                 910

Ile Thr Val Glu Glu Ser Leu Ile Tyr Ser Ala Trp Leu Arg Leu Val
        915                 920                 925

Pro Glu Ile Asn Pro Gln Thr Lys Ile Arg Phe Val Lys Gln Val Leu
    930                 935                 940

Glu Thr Ile Glu Leu Glu Glu Ile Lys Asp Ala Leu Val Gly Val Ala
945                 950                 955                 960

Gly Val Ser Gly Leu Ser Thr Glu Gln Arg Lys Arg Leu Thr Val Ala
                965                 970                 975

Val Glu Leu Val Ala Asn Pro Ser Ile Ile Phe Met Asp Glu Pro Thr
            980                 985                 990

Thr Gly Leu Asp Ala Arg Ala Ala  Ala Ile Val Met Arg  Ala Val Lys
        995                 1000                1005

Asn Val  Ala Glu Thr Gly Arg  Thr Ile Val Cys Thr  Ile His Gln
    1010                1015                1020

Pro Ser  Ile His Ile Phe Glu  Ala Phe Asp Glu Leu  Val Leu Leu
    1025                1030                1035

Lys Arg  Gly Gly Arg Met Ile  Tyr Ser Gly Pro Leu  Gly Gln His
    1040                1045                1050

Ser Ser  Cys Val Ile Glu Tyr  Phe Gln Asn Ile Pro  Gly Val Ala
    1055                1060                1065

Lys Ile  Arg Asp Lys Tyr Asn  Pro Ala Thr Trp Met  Leu Glu Val
    1070                1075                1080

Thr Ser  Glu Ser Val Glu Thr  Glu Leu Asp Met Asp  Phe Ala Lys
    1085                1090                1095

Ile Tyr  Asn Glu Ser Asp Leu  Tyr Lys Asn Asn Ser  Glu Leu Val
    1100                1105                1110

Lys Glu  Leu Ser Lys Pro Asp  His Gly Ser Ser Asp  Leu His Phe
    1115                1120                1125

Lys Arg  Thr Phe Ala Gln Asn  Trp Trp Glu Gln Phe  Lys Ser Cys
    1130                1135                1140

Leu Trp  Lys Met Ser Leu Ser  Tyr Trp Arg Ser Pro  Ser Tyr Asn
    1145                1150                1155

Leu Met  Arg Ile Gly His Thr  Phe Ile Ser Ser Phe  Ile Phe Gly
    1160                1165                1170

Leu Leu  Phe Trp Asn Gln Gly  Lys Lys Ile Asp Thr  Gln Gln Asn
    1175                1180                1185

Leu Phe  Thr Val Leu Gly Ala  Ile Tyr Gly Leu Val  Leu Phe Val
    1190                1195                1200

Gly Ile  Asn Asn Cys Thr Ser  Ala Leu Gln Tyr Phe  Glu Thr Glu
    1205                1210                1215

Arg Asn  Val Met Tyr Arg Glu  Arg Phe Ala Gly Met  Tyr Ser Ala
    1220                1225                1230
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Phe Ala  Tyr Ala Leu Ala Gln  Val Val Thr Glu Ile  Pro Tyr Ile
    1235                 1240                 1245

Phe Ile  Gln Ser Ala Glu Phe  Val Ile Val Ile Tyr  Pro Met Ile
    1250                 1255                 1260

Gly Phe  Tyr Ala Ser Phe Ser  Lys Val Phe Trp Ser  Leu Tyr Ala
    1265                 1270                 1275

Met Phe  Cys Asn Leu Leu Cys  Phe Asn Tyr Leu Ala  Met Phe Leu
    1280                 1285                 1290

Ile Ser  Ile Thr Pro Asn Phe  Met Val Ala Ala Ile  Leu Gln Ser
    1295                 1300                 1305

Leu Phe  Phe Thr Thr Phe Asn  Ile Phe Ala Gly Phe  Leu Ile Pro
    1310                 1315                 1320

Lys Pro  Gln Ile Pro Lys Trp  Trp Val Trp Phe Tyr  Tyr Ile Thr
    1325                 1330                 1335

Pro Thr  Ser Trp Thr Leu Asn  Leu Phe Phe Ser Ser  Gln Tyr Gly
    1340                 1345                 1350

Asp Ile  His Gln Lys Ile Asn  Ala Phe Gly Glu Thr  Lys Thr Val
    1355                 1360                 1365

Ala Ser  Phe Leu Glu Asp Tyr  Phe Gly Phe His His  Asp Arg Leu
    1370                 1375                 1380

Met Ile  Thr Ala Ile Ile Leu  Ile Ala Phe Pro Ile  Ala Leu Ala
    1385                 1390                 1395

Thr Met  Tyr Ala Phe Phe Val  Ala Lys Leu Asn Phe  Gln Lys Arg
    1400                 1405                 1410


<210> SEQ ID NO 49
<211> LENGTH: 1450
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 49

Met Ala His Met Val Gly Ala Asp Asp Ile Glu Ser Leu Arg Val Glu
1               5                  10                  15

Leu Ala Glu Ile Gly Arg Ser Ile Arg Ser Ser Phe Arg Arg His Thr
            20                  25                  30

Ser Ser Phe Arg Ser Ser Ser Ser Ile Tyr Glu Val Glu Asn Asp Gly
        35                  40                  45

Asp Val Asn Asp His Asp Ala Glu Tyr Ala Leu Gln Trp Ala Glu Ile
    50                  55                  60

Glu Arg Leu Pro Thr Val Lys Arg Met Arg Ser Thr Leu Leu Asp Asp
65                  70                  75                  80

Gly Asp Glu Ser Met Thr Glu Lys Gly Arg Arg Val Val Asp Val Thr
                85                  90                  95

Lys Leu Gly Ala Val Glu Arg His Leu Met Ile Glu Lys Leu Ile Lys
            100                 105                 110

His Ile Glu Asn Asp Asn Leu Lys Leu Leu Lys Lys Ile Arg Arg Arg
        115                 120                 125

Ile Asp Arg Val Gly Met Glu Leu Pro Thr Ile Glu Val Arg Tyr Glu
    130                 135                 140

Ser Leu Lys Val Val Ala Glu Cys Glu Val Val Glu Gly Lys Ala Leu
145                 150                 155                 160

Pro Thr Leu Trp Asn Thr Ala Lys Arg Val Leu Ser Glu Leu Val Lys
                165                 170                 175

Leu Thr Gly Ala Lys Thr His Glu Ala Lys Ile Asn Ile Ile Asn Asp
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           180                 185                 190

Val Asn Gly Ile Ile Lys Pro Gly Arg Leu Thr Leu Leu Leu Gly Pro
        195                 200                 205

Pro Ser Cys Gly Lys Thr Thr Leu Leu Lys Ala Leu Ser Gly Asn Leu
    210                 215                 220

Glu Asn Asn Leu Lys Cys Ser Gly Glu Ile Ser Tyr Asn Gly His Arg
225                 230                 235                 240

Leu Asp Glu Phe Val Pro Gln Lys Thr Ser Ala Tyr Ile Ser Gln Tyr
                245                 250                 255

Asp Leu His Ile Ala Glu Met Thr Val Arg Glu Thr Val Asp Phe Ser
            260                 265                 270

Ala Arg Cys Gln Gly Val Gly Ser Arg Thr Asp Ile Met Met Glu Val
        275                 280                 285

Ser Lys Arg Glu Lys Glu Lys Gly Ile Ile Pro Asp Thr Glu Val Asp
    290                 295                 300

Ala Tyr Met Lys Ala Ile Ser Val Glu Gly Leu Gln Arg Ser Leu Gln
305                 310                 315                 320

Thr Asp Tyr Ile Leu Lys Ile Leu Gly Leu Asp Ile Cys Ala Glu Ile
                325                 330                 335

Leu Ile Gly Asp Val Met Arg Arg Gly Ile Ser Gly Gly Gln Lys Lys
            340                 345                 350

Arg Leu Thr Thr Ala Glu Met Ile Val Gly Pro Thr Lys Ala Leu Phe
        355                 360                 365

Met Asp Glu Ile Thr Asn Gly Leu Asp Ser Ser Thr Ala Phe Gln Ile
    370                 375                 380

Val Lys Ser Leu Gln Gln Phe Ala His Ile Ser Ser Ala Thr Val Leu
385                 390                 395                 400

Val Ser Leu Leu Gln Pro Ala Pro Glu Ser Tyr Asp Leu Phe Asp Asp
                405                 410                 415

Ile Met Leu Met Ala Lys Gly Arg Ile Val Tyr His Gly Pro Arg Gly
            420                 425                 430

Glu Val Leu Asn Phe Phe Glu Asp Cys Gly Phe Arg Cys Pro Glu Arg
        435                 440                 445

Lys Gly Val Ala Asp Phe Leu Gln Glu Val Ile Ser Lys Lys Asp Gln
    450                 455                 460

Ala Gln Tyr Trp Trp His Glu Asp Leu Pro Tyr Ser Phe Val Ser Val
465                 470                 475                 480

Glu Met Leu Ser Lys Lys Phe Lys Asp Leu Ser Ile Gly Lys Lys Ile
                485                 490                 495

Glu Asp Thr Leu Ser Lys Pro Tyr Asp Arg Ser Lys Ser His Lys Asp
            500                 505                 510

Ala Leu Ser Phe Ser Val Tyr Ser Leu Pro Asn Trp Glu Leu Phe Ile
        515                 520                 525

Ala Cys Ile Ser Arg Glu Tyr Leu Leu Met Lys Arg Asn Tyr Phe Val
    530                 535                 540

Tyr Ile Phe Lys Thr Ala Gln Leu Val Met Ala Ala Phe Ile Thr Met
545                 550                 555                 560

Thr Val Phe Ile Arg Thr Arg Met Gly Ile Asp Ile Ile His Gly Asn
                565                 570                 575

Ser Tyr Met Ser Ala Leu Phe Phe Ala Leu Ile Ile Leu Leu Val Asp
            580                 585                 590

Gly Phe Pro Glu Leu Ser Met Thr Ala Gln Arg Leu Ala Val Phe Tyr
        595                 600                 605
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Lys Gln Lys Gln Leu Cys Phe Tyr Pro Ala Trp Ala Tyr Ala Ile Pro
    610                 615                 620

Ala Thr Val Leu Lys Val Pro Leu Ser Phe Phe Glu Ser Leu Val Trp
625                 630                 635                 640

Thr Cys Leu Ser Tyr Tyr Val Ile Gly Tyr Thr Pro Glu Ala Ser Arg
                645                 650                 655

Phe Phe Lys Gln Phe Ile Leu Leu Phe Ala Val His Phe Thr Ser Ile
            660                 665                 670

Ser Met Phe Arg Cys Leu Ala Ala Ile Phe Gln Thr Val Val Ala Ser
        675                 680                 685

Ile Thr Ala Gly Ser Phe Gly Ile Leu Phe Thr Phe Val Phe Ala Gly
    690                 695                 700

Phe Val Ile Pro Pro Pro Ser Met Pro Ala Trp Leu Lys Trp Gly Phe
705                 710                 715                 720

Trp Ala Asn Pro Leu Ser Tyr Gly Glu Ile Gly Leu Ser Val Asn Glu
                725                 730                 735

Phe Leu Ala Pro Arg Trp Asn Gln Met Gln Pro Asn Asn Phe Thr Leu
            740                 745                 750

Gly Arg Thr Ile Leu Gln Thr Arg Gly Met Asp Tyr Asn Gly Tyr Met
        755                 760                 765

Tyr Trp Val Ser Leu Cys Ala Leu Leu Gly Phe Thr Val Leu Phe Asn
    770                 775                 780

Ile Ile Phe Thr Leu Ala Leu Thr Phe Leu Lys Ser Pro Thr Ser Ser
785                 790                 795                 800

Arg Ala Met Ile Ser Gln Asp Lys Leu Ser Glu Leu Gln Gly Thr Glu
                805                 810                 815

Lys Ser Thr Glu Asp Ser Ser Val Arg Lys Lys Thr Thr Asp Ser Pro
            820                 825                 830

Val Lys Thr Glu Glu Glu Asp Lys Met Val Leu Pro Phe Lys Pro Leu
        835                 840                 845

Thr Val Thr Phe Gln Asp Leu Asn Tyr Phe Val Asp Met Pro Val Glu
    850                 855                 860

Met Arg Asp Gln Gly Tyr Asp Gln Lys Lys Leu Gln Leu Leu Ser Asp
865                 870                 875                 880

Ile Thr Gly Ala Phe Arg Pro Gly Ile Leu Thr Ala Leu Met Gly Val
                885                 890                 895

Ser Gly Ala Gly Lys Thr Thr Leu Leu Asp Val Leu Ala Gly Arg Lys
            900                 905                 910

Thr Ser Gly Tyr Ile Glu Gly Asp Ile Arg Ile Ser Gly Phe Pro Lys
        915                 920                 925

Val Gln Glu Thr Phe Ala Arg Val Ser Gly Tyr Cys Glu Gln Thr Asp
    930                 935                 940

Ile His Ser Pro Asn Ile Thr Val Glu Glu Ser Val Ile Tyr Ser Ala
945                 950                 955                 960

Trp Leu Arg Leu Ala Pro Glu Ile Asp Ala Thr Thr Lys Thr Lys Phe
                965                 970                 975

Val Lys Gln Val Leu Glu Thr Ile Glu Leu Asp Glu Ile Lys Asp Ser
            980                 985                 990

Leu Val Gly Val Thr Gly Val Ser  Gly Leu Ser Thr Glu  Gln Arg Lys
        995                 1000                1005

Arg Leu  Thr Ile Ala Val Glu  Leu Val Ala Asn Pro  Ser Ile Ile
    1010                1015                1020
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Phe Met  Asp Glu Pro Thr Thr  Gly Leu Asp Ala Arg  Ala Ala Ala
    1025                1030                1035

Ile Val  Met Arg Ala Val Lys  Asn Val Ala Asp Thr  Gly Arg Thr
    1040                1045                1050

Ile Val  Cys Thr Ile His Gln  Pro Ser Ile Asp Ile  Phe Glu Ala
    1055                1060                1065

Phe Asp  Glu Leu Val Leu Leu  Lys Arg Gly Gly Arg  Met Ile Tyr
    1070                1075                1080

Thr Gly  Pro Leu Gly Gln His  Ser Arg His Ile Ile  Glu Tyr Phe
    1085                1090                1095

Glu Ser  Val Pro Glu Ile Pro  Lys Ile Lys Asp Asn  His Asn Pro
    1100                1105                1110

Ala Thr  Trp Met Leu Asp Val  Ser Ser Gln Ser Val  Glu Ile Glu
    1115                1120                1125

Leu Gly  Val Asp Phe Ala Lys  Ile Tyr His Asp Ser  Ala Leu Tyr
    1130                1135                1140

Lys Arg  Asn Ser Glu Leu Val  Lys Gln Leu Ser Gln  Pro Asp Ser
    1145                1150                1155

Gly Ser  Ser Asp Ile Gln Phe  Lys Arg Thr Phe Ala  Gln Ser Trp
    1160                1165                1170

Trp Gly  Gln Phe Lys Ser Ile  Leu Trp Lys Met Asn  Leu Ser Tyr
    1175                1180                1185

Trp Arg  Ser Pro Ser Tyr Asn  Leu Met Arg Met Met  His Thr Leu
    1190                1195                1200

Val Ser  Ser Leu Ile Phe Gly  Ala Leu Phe Trp Lys  Gln Gly Gln
    1205                1210                1215

Asn Leu  Asp Thr Gln Gln Ser  Met Phe Thr Val Phe  Gly Ala Ile
    1220                1225                1230

Tyr Gly  Leu Val Leu Phe Leu  Gly Ile Asn Asn Cys  Ala Ser Ala
    1235                1240                1245

Leu Gln  Tyr Phe Glu Thr Glu  Arg Asn Val Met Tyr  Arg Glu Arg
    1250                1255                1260

Phe Ala  Gly Met Tyr Ser Ala  Thr Ala Tyr Ala Leu  Gly Gln Val
    1265                1270                1275

Val Thr  Glu Ile Pro Tyr Ile  Phe Ile Gln Ala Ala  Glu Phe Val
    1280                1285                1290

Ile Val  Thr Tyr Pro Met Ile  Gly Phe Tyr Pro Ser  Ala Tyr Lys
    1295                1300                1305

Val Phe  Trp Ser Leu Tyr Ser  Met Phe Cys Ser Leu  Leu Thr Phe
    1310                1315                1320

Asn Tyr  Leu Ala Met Phe Leu  Val Ser Ile Thr Pro  Asn Phe Met
    1325                1330                1335

Val Ala  Ala Ile Leu Gln Ser  Leu Phe Tyr Val Gly  Phe Asn Leu
    1340                1345                1350

Phe Ser  Gly Phe Leu Ile Pro  Gln Thr Gln Val Pro  Gly Trp Trp
    1355                1360                1365

Ile Trp  Leu Tyr Tyr Leu Thr  Pro Thr Ser Trp Thr  Leu Asn Gly
    1370                1375                1380

Phe Ile  Ser Ser Gln Tyr Gly  Asp Ile His Glu Glu  Ile Asn Val
    1385                1390                1395

Phe Gly  Gln Ser Thr Thr Val  Ala Arg Phe Leu Lys  Asp Tyr Phe
    1400                1405                1410

Gly Phe  His His Asp Leu Leu  Ala Val Thr Ala Val  Val Gln Ile
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    1415                1420                1425

Ala Phe  Pro Ile Ala Leu Ala  Ser Met Phe Ala Phe  Phe Val Gly
    1430                1435                1440

Lys Leu  Asn Phe Gln Arg Arg
    1445                1450


<210> SEQ ID NO 50
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Conserved domain of PDR ABC transporters
      present in Lr34

<400> SEQUENCE: 50

Gly Pro Pro Gly Cys Gly Lys Ser
1               5


<210> SEQ ID NO 51
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Conserved domain of PDR ABC transporters
      present in Lr34

<400> SEQUENCE: 51

Gly Val Ser Gly Ala Gly Lys Thr
1               5


<210> SEQ ID NO 52
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Conserved domain of PDR ABC transporters
      present in Lr34

<400> SEQUENCE: 52

Ile Ser Gly Gly Gln Lys Lys Arg Leu Thr Thr Ala
1               5                   10


<210> SEQ ID NO 53
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Conserved domain of PDR ABC transporters
      present in Lr34

<400> SEQUENCE: 53

Leu Ser Met Glu Gln Arg Lys Arg Leu Thr Ile Ala
1               5                   10


<210> SEQ ID NO 54
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Conserved domain of PDR ABC transporters
      present in Lr34

<400> SEQUENCE: 54

Ala Tyr Phe Met Asp
1               5
```

-continued

<210> SEQ ID NO 55
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Conserved domain of PDR ABC transporters present in Lr34

<400> SEQUENCE: 55

Ile Ile Leu Met Asp
1 5

<210> SEQ ID NO 56
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Walker A consensus sequence
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa = Any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa = Any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa = Any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa = Ser or Thr

<400> SEQUENCE: 56

Gly Xaa Xaa Xaa Xaa Gly Lys Xaa
1 5

<210> SEQ ID NO 57
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Walker B consensus sequence
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa = Phe, Ile, Trp, Leu, Val, Met, Tyr or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = Phe, Ile, Trp, Leu, Val, Met, Tyr or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa = Phe, Ile, Trp, Leu, Val, Met, Tyr or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa = Phe, Ile, Trp, Leu, Val, Met, Tyr or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa = Asp or Glu

<400> SEQUENCE: 57

Xaa Xaa Xaa Xaa Xaa
1 5

-continued

<210> SEQ ID NO 58
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ABC signature consensus sequence
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa = Leu, Ile, Val, Met, Phe or Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa = Ser, Gly or Met
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa = Gly or Glu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa = Any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa = Any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa = Any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa = Arg, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa = Leu, Ile, Val, Met, Tyr or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa = Any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa = Leu, Ile, Val, Met, Phe or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: Xaa = Ala or Gly

<400> SEQUENCE: 58

Xaa Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10

<210> SEQ ID NO 59
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PDR signature 1

<400> SEQUENCE: 59

Leu Leu Leu Gly Pro Pro
1 5

<210> SEQ ID NO 60
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PDR signature 2

<400> SEQUENCE: 60

-continued

Gly Leu Asp Ser Ser Thr
1 5

<210> SEQ ID NO 61
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PDR signature 3
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa = Ala or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa = Ala or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa = Met or Ile

<400> SEQUENCE: 61

Gly Leu Asp Xaa Arg Xaa Ala Ala Ile Val Xaa Arg
1 5 10

<210> SEQ ID NO 62
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PDR signature 4

<400> SEQUENCE: 62

Val Cys Thr Ile His Gln Pro Ser
1 5

<210> SEQ ID NO 63
<211> LENGTH: 1405
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 63

Met Glu Gly Leu Ala Arg Glu Thr Asn Pro Ser Ser His His Gln Asp
1 5 10 15

Phe Ala Ser Cys Ala Ser Asp Glu Arg Pro Asp Glu Pro Glu Leu Glu
20 25 30

Leu Ala Ser Arg Arg Arg Gln Asn Gly Ala Gly Asn Asn Glu His Val
35 40 45

Ser Glu Asn Met Leu Leu Asp Ser Ser Lys Phe Gly Ala Leu Lys Arg
50 55 60

Arg Glu Phe Phe Asn Asn Leu Leu Lys Asn Leu Glu Asp Asp His Pro
65 70 75 80

Arg Phe Leu Arg Arg Gln Lys Glu Arg Ile Asp Arg Val Asp Val Lys
85 90 95

Leu Pro Ala Ile Glu Val Arg Tyr Asn Asn Leu Phe Val Glu Ala Glu
100 105 110

Cys Arg Val Thr Lys Gly Asn His Leu Pro Ser Leu Trp Asn Ser Thr
115 120 125

Lys Gly Ala Phe Ser Gly Leu Val Lys Leu Leu Gly Phe Glu Thr Glu
130 135 140

Arg Ala Lys Thr Asn Val Leu Glu Asp Val Ser Gly Ile Ile Lys Pro
145 150 155 160

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Cys Arg Leu Thr Leu Leu Leu Gly Pro Pro Gly Cys Gly Lys Ser Thr
                165                 170                 175

Leu Leu Arg Ala Leu Ala Gly Lys Leu Asp Lys Ser Leu Lys Val Thr
            180                 185                 190

Gly Asp Ile Ser Tyr Asn Cys Tyr Glu Leu His Glu Phe Val Pro Glu
        195                 200                 205

Lys Thr Ala Val Tyr Ile Asn Gln His Asp Leu His Ile Ala Glu Met
    210                 215                 220

Thr Val Arg Glu Thr Leu Asp Phe Ser Ala Gln Cys Gln Gly Val Gly
225                 230                 235                 240

Arg Arg Pro Lys Ile Leu Lys Glu Val Asn Thr Arg Glu Ser Val Ala
                245                 250                 255

Gly Ile Ile Pro Asp Ala Asp Ile Asp Leu Tyr Met Lys Val Val Ala
            260                 265                 270

Val Glu Ala Ser Glu Arg Ser Leu Gln Thr Asp Tyr Ile Leu Lys Ile
        275                 280                 285

Met Gly Leu Glu Thr Cys Ala Asp Thr Met Val Gly Asp Ala Met Arg
    290                 295                 300

Arg Gly Ile Ser Gly Gly Gln Lys Lys Arg Leu Thr Thr Ala Glu Met
305                 310                 315                 320

Ile Val Gly Pro Ala Lys Ala Tyr Phe Met Asp Glu Ile Ser Asn Gly
                325                 330                 335

Leu Asp Ser Ser Thr Thr Phe Gln Ile Ile Asn Cys Phe Gln Gln Leu
            340                 345                 350

Thr Asn Ile Ser Glu Tyr Thr Met Val Ile Ser Leu Leu Gln Pro Thr
        355                 360                 365

Pro Glu Val Phe Asp Leu Phe Asp Asp Leu Ile Leu Met Ala Glu Gly
    370                 375                 380

Lys Ile Ile Tyr His Gly Pro Arg Asn Glu Ala Leu Asn Phe Phe Glu
385                 390                 395                 400

Glu Cys Gly Phe Lys Cys Pro Glu Arg Lys Ala Ala Ala Asp Phe Leu
                405                 410                 415

Gln Glu Ile Leu Ser Arg Lys Asp Gln Glu Gln Tyr Trp Leu Gly Pro
            420                 425                 430

His Glu Ser Tyr Arg Tyr Ile Ser Pro His Glu Leu Ser Ser Met Phe
        435                 440                 445

Lys Glu Asn His Arg Gly Arg Lys Leu His Glu Gln Ser Val Pro Pro
    450                 455                 460

Lys Ser Gln Phe Gly Lys Glu Ala Leu Ala Phe Asn Lys Tyr Ser Leu
465                 470                 475                 480

Arg Lys Leu Glu Met Phe Lys Ala Cys Gly Ala Arg Glu Ala Leu Leu
                485                 490                 495

Met Lys Arg Asn Met Phe Val Tyr Val Phe Lys Thr Gly Gln Leu Ala
            500                 505                 510

Ile Ile Ala Leu Val Thr Met Ser Val Phe Leu Arg Thr Arg Met Thr
        515                 520                 525

Ile Ser Phe Thr His Ala Asn Tyr Tyr Met Gly Ala Leu Phe Phe Ser
    530                 535                 540

Ile Phe Met Ile Met Leu Asn Gly Ile Pro Glu Met Ser Met Gln Ile
545                 550                 555                 560

Gly Arg Leu Pro Ser Phe Tyr Lys Gln Lys Ser Tyr Tyr Phe Tyr Ser
                565                 570                 575
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Ser Trp Ala Tyr Ala Ile Pro Ala Ser Val Leu Lys Val Pro Val Ser
            580                 585                 590

Ile Leu Asp Ser Leu Val Trp Ile Ser Ile Thr Tyr Tyr Gly Ile Gly
        595                 600                 605

Tyr Thr Pro Thr Val Ser Arg Phe Phe Cys Gln Phe Leu Ile Leu Cys
    610                 615                 620

Leu Leu His His Ser Val Thr Ser Gln Tyr Arg Phe Ile Ala Ser Tyr
625                 630                 635                 640

Phe Gln Thr Pro Ile Val Ser Phe Phe Tyr Leu Phe Leu Ala Leu Thr
                645                 650                 655

Val Phe Leu Thr Phe Gly Gly Phe Ile Leu Pro Lys Thr Ser Met Pro
            660                 665                 670

Glu Trp Leu Asn Trp Gly Phe Trp Ile Ser Pro Met Ala Tyr Ala Glu
        675                 680                 685

Ile Ser Ile Val Ile Asn Glu Phe Leu Ala Pro Arg Trp Gln Lys Glu
    690                 695                 700

Ser Ile Gln Asn Ile Thr Ile Gly Asn Gln Ile Leu Val Asn His Gly
705                 710                 715                 720

Leu Tyr Tyr Ser Trp His Phe Tyr Trp Ile Ser Phe Gly Ala Leu Leu
                725                 730                 735

Gly Ser Ile Leu Leu Phe Tyr Ile Ala Phe Gly Leu Ala Leu Asp Tyr
            740                 745                 750

Arg Thr Pro Thr Glu Glu Tyr His Gly Ser Arg Pro Thr Lys Ser Leu
        755                 760                 765

Cys Gln Gln Gln Glu Lys Asp Ser Thr Ile Gln Asn Glu Ser Asp Asp
    770                 775                 780

Gln Ser Asn Ile Ser Lys Ala Lys Met Thr Ile Pro Thr Met His Leu
785                 790                 795                 800

Pro Ile Thr Phe His Asn Leu Asn Tyr Tyr Ile Asp Thr Pro Pro Glu
                805                 810                 815

Met Leu Lys Gln Gly Tyr Pro Thr Arg Arg Leu Arg Leu Leu Asn Asn
            820                 825                 830

Ile Thr Gly Ala Leu Arg Pro Gly Val Leu Ser Ala Leu Met Gly Val
        835                 840                 845

Ser Gly Ala Gly Lys Thr Thr Leu Leu Asp Val Leu Ala Gly Arg Lys
    850                 855                 860

Thr Gly Gly Tyr Ile Glu Gly Asp Ile Arg Ile Gly Gly Tyr Pro Lys
865                 870                 875                 880

Val Gln Glu Thr Phe Val Arg Ile Leu Gly Tyr Cys Glu Gln Val Asp
                885                 890                 895

Ile His Ser Pro Gln Leu Thr Val Glu Glu Ser Val Thr Tyr Ser Ala
            900                 905                 910

Trp Leu Arg Leu Pro Ser His Val Asp Lys Gln Thr Arg Ser Lys Phe
        915                 920                 925

Val Ala Glu Val Leu Glu Thr Val Glu Leu Asp Gln Ile Lys Asp Val
    930                 935                 940

Leu Val Gly Ser Pro Gln Lys Asn Gly Leu Ser Met Glu Gln Arg Lys
945                 950                 955                 960

Arg Leu Thr Ile Ala Val Glu Leu Val Ser Asn Pro Ser Ile Ile Leu
                965                 970                 975

Met Asp Glu Pro Thr Thr Gly Leu Asp Thr Arg Ser Ala Ala Ile Val
            980                 985                 990

Ile Arg Ala Val Lys Asn Ile Cys  Glu Thr Gly Arg Thr  Val Val Cys
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        995                 1000                1005

Thr Ile  His Gln Pro Ser Thr  Glu Ile Phe Glu Ala  Phe Asp Glu
    1010                1015                1020

Leu Ile  Leu Met Lys Thr Gly  Gly Lys Thr Ile Tyr  Asn Gly Pro
    1025                1030                1035

Ile Gly  Glu Arg Ser Cys Lys  Val Ile Glu Tyr Phe  Glu Lys Ile
    1040                1045                1050

Ser Gly  Val Pro Lys Ile Lys  Ser Asn Cys Asn Pro  Ala Thr Trp
    1055                1060                1065

Met Met  Asp Val Thr Ser Thr  Ser Met Glu Val Gln  His Asn Met
    1070                1075                1080

Asp Phe  Ala Ile Leu Tyr Glu  Glu Ser Ser Leu His  Arg Glu Ala
    1085                1090                1095

Glu Asp  Leu Val Glu Gln Leu  Ser Ile Pro Leu Pro  Asn Ser Glu
    1100                1105                1110

Asn Leu  Arg Phe Ser His Ser  Phe Ala Gln Asn Gly  Trp Ile Gln
    1115                1120                1125

Leu Lys  Ala Cys Leu Trp Lys  Gln Asn Ile Thr Tyr  Trp Arg Ser
    1130                1135                1140

Pro Gln  Tyr Asn Leu Arg Arg  Ile Met Met Thr Val  Ile Ser Ala
    1145                1150                1155

Leu Ile  Tyr Gly Val Leu Phe  Trp Lys His Ala Lys  Val Leu Asn
    1160                1165                1170

Asn Glu  Gln Asp Met Leu Ser  Val Phe Gly Ala Met  Tyr Leu Gly
    1175                1180                1185

Phe Thr  Thr Ile Gly Ala Tyr  Asn Asp Gln Thr Ile  Ile Pro Phe
    1190                1195                1200

Ser Thr  Thr Glu Arg Ile Val  Met Tyr Arg Glu Lys  Phe Ala Gly
    1205                1210                1215

Met Tyr  Ser Ser Trp Ser Tyr  Ser Phe Ala Gln Ala  Phe Ile Glu
    1220                1225                1230

Ile Pro  Tyr Val Phe Ile Gln  Val Val Leu Tyr Thr  Leu Ile Val
    1235                1240                1245

Tyr Pro  Ser Thr Gly Tyr Tyr  Trp Thr Ala His Lys  Phe Leu Trp
    1250                1255                1260

Phe Phe  Tyr Thr Thr Phe Cys  Ser Ile Leu Ser Tyr  Val Tyr Val
    1265                1270                1275

Gly Leu  Leu Leu Val Ser Ile  Thr Pro Asn Val Gln  Val Ala Thr
    1280                1285                1290

Ile Leu  Ala Ser Phe Phe Asn  Thr Met Gln Thr Leu  Phe Ser Gly
    1295                1300                1305

Phe Ile  Leu Pro Ala Pro Thr  Leu Gln Gln Ile Pro  Lys Trp Trp
    1310                1315                1320

Thr Trp  Leu Tyr Tyr Leu Thr  Pro Thr Ser Trp Ala  Leu Asn Ala
    1325                1330                1335

Leu Leu  Thr Ser Gln Tyr Gly  Asn Ile Glu Lys Glu  Val Lys Ala
    1340                1345                1350

Phe Gly  Glu Thr Lys Ser Val  Ser Ile Phe Leu Asn  Asp Tyr Phe
    1355                1360                1365

Gly Phe  His Gln Asp Lys Leu  Ser Ile Val Ala Thr  Val Leu Val
    1370                1375                1380
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Ala Phe  Pro Phe Val Leu Ile  Ile Leu Phe Ser Leu  Ser Ile Glu
    1385                1390                1395

Lys Leu  Asn Phe Gln Lys Arg
    1400                1405


<210> SEQ ID NO 64
<211> LENGTH: 4218
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 64

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gcgagtgacg agcgcccgga tgagcccgag ttggaattgg catcgcgacg gcgccagaat    120

ggtgctggaa acaacgagca tgtgagtgag aacatgctgc ttgacagcag caagtttgga    180

gctctcaaga ggcgtgagtt cttcaacaac ctgctaaaga acctcgaaga cgaccacccc    240

cgctttctgc gcagacaaaa ggaaagaatt gacagggttg atgtcaagtt gccagcaata    300

gaggtgaggt ataataatct gtttgtggaa gcagagtgca gagttactaa aggaaatcac    360

ctgccgtctc tatggaatag taccaaaggt gccttctcgg gcctcgtgaa gttgctaggc    420

ttcgaaacgg aaagagcaaa aaccaacgtt ctagaagatg tcagtggaat catcaaaccc    480

tgcagattga ctcttctact gggacctcct ggatgtggca aaagcactct gttgcgagct    540

cttgccggga aactagataa atctctaaag gtaacagggg atatctctta taattgttat    600

gaacttcatg aatttgtacc tgagaaaaca gctgtgtata tcaaccaaca tgatctgcac    660

atagctgaga tgactgtgag ggaaacttta gacttctcag cccagtgcca aggtgttgga    720

agaagaccaa aaatactcaa ggaggtgaac acaagggaga gtgtggctgg gatcatacct    780

gatgcggaca tcgatctata catgaaggta gtagcagttg aagcttcaga gcgaagccta    840

cagacagatt atattttgaa gatcatgggg ctagagacat gcgcagacac gatggttggg    900

gatgcaatga gaagaggaat atcaggggggg cagaagaaaa gattaaccac agccgagatg    960

attgtgggac ccgcaaaagc atactttatg gatgaaatat caaatggtct ggatagctct   1020

accacttttc aaataatcaa ttgtttccag caactgacaa acatcagcga gtacacgatg   1080

gttatttcac ttcttcaacc aacacctgag gtatttgatc ttttcgatga cctcatacta   1140

atggcagaag ggaagattat ctaccatggc cctcgaaatg aagccctcaa ttttttgag   1200

gagtgtgggt tcaaatgccc agaaagaaaa gcggcagctg actttctcca agagatcttg   1260

tccaggaagg accaagaaca gtactggttg ggtccacatg aatcatacag atatatctca   1320

cctcatgaat tatcaagcat gttcaaggag aatcacaggg ggagaaaact acatgaacaa   1380

agtgtacctc ccaaaagcca gttcggcaag gaagctttag cattcaataa gtattcgcta   1440

cgaaaactgg aaatgttcaa agcctgtgga gcaagggaag cactcctaat gaaaaggaat   1500

atgtttgttt atgtcttcaa aacaggccag cttgccatta ttgcactcgt aacaatgtct   1560

gtattccttc gaactcgcat gacaataagt ttcactcatg caaattacta tatgggagca   1620

ttatttttt ccatcttcat gattatgcta aatggcatac cagagatgag catgcagatt   1680

gggagactcc caagttttta caaacaaaag agctactatt tctattcatc atgggcatat   1740

gcaataccag cttcagtcct aaaggtccct gtttccatac tggattcgct tgtatggata   1800

tctatcacat attatggtat tggttataca cctactgttt caaggttctt ctgccagttt   1860

ctgatacttt gtcttctcca tcattcagtc acctcgcagt atcgatttat tgcttcatac   1920
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ttccaaacac ctattgtgtc tttcttctac ctttttcttg ctctaacagt attccttaca	1980
ttcggaggct tcattcttcc caagacctcc atgccagaat ggctaaactg gggattttgg	2040
atatctccaa tggcatatgc agaaatcagc atagttatta acgagttctt ggcaccaaga	2100
tggcagaagg aaagtattca aaacataaca attgggaacc aaatcctggt taatcacggc	2160
ctatattaca gttggcattt ttattggata tcctttggag ccttgcttgg atctattctt	2220
ttattttata tcgcttttgg attggcacta gattacagaa cacctacaga agaatatcat	2280
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gcaggaagga aaacaggagg ttatattgaa ggggacataa gaataggtgg atatcccaag	2640
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aatttccaga agaggtaa	4218

The invention claimed is:

1. A transgenic cereal plant which has integrated into its genome an exogenous polynucleotide encoding an Lr34 adult plant pathogen resistance polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence set forth as SEQ ID NO:1, wherein the adult plant pathogen resistance polypeptide provides enhanced resistance to a plant pathogen when compared to an isogenic cereal plant lacking the exogenous polynucleotide, and wherein the pathogen is one or more of the following pathogens: *Bipolaris sorokiniana, Erysiphe graminis* f. sp. *tritici, Puccinia graminis* f. sp. *tritici, Puccinia striiformis* and *Puccinia recondite* f. sp. *tritici.*

2. The transgenic cereal plant of claim **1** wherein the plant is a wheat plant.

3. The transgenic cereal plant of claim **1**, wherein the amino acid sequence of the Lr34 adult plant pathogen resistance polypeptide is at least 98% identical to the amino acid sequence set forth as SEQ ID NO:1.

4. The transgenic cereal plant of claim **1**, wherein the amino acid sequence of the Lr34 adult plant pathogen resistance polypeptide comprises the amino acid sequence set forth as SEQ ID NO:1.

5. The transgenic cereal plant of claim **4** wherein the plant is a wheat plant.

6. A transgenic cereal seed which has integrated into its genome an exogenous polynucleotide encoding an Lr34 adult plant pathogen resistance polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence set forth as SEQ ID NO:1, wherein the adult plant pathogen resistance polypeptide provides enhanced resistance to a cereal plant produced from the seed to a plant pathogen when compared to an isogenic cereal plant lacking the exogenous polynucleotide, and wherein the pathogen is one or more of the following pathogens: *Bipolaris sorokiniana, Erysiphe graminis* f. sp. *tritici, Puccinia graminis* f. sp. *tritici, Puccinia striiformis* and *Puccinia recondita* f. sp. *tritici.*

7. The transgenic cereal seed of claim **6** wherein the seed is a wheat seed.

8. The transgenic cereal seed of claim **6**, wherein the amino acid sequence of the Lr34 adult plant pathogen resistance polypeptide is at least 98% identical to the amino acid sequence set forth as SEQ ID NO:1.

9. The transgenic cereal seed of claim **6**, wherein the amino acid sequence of the Lr34 adult plant pathogen resistance polypeptide comprises the amino acid sequence set forth as SEQ ID NO:1.

10. The transgenic cereal seed of claim **9** wherein the seed is a wheat seed.

11. A method of producing a transgenic cereal plant which has integrated into its genome an exogenous polynucleotide encoding an Lr34 adult plant pathogen resistance polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence set forth as SEQ ID NO:1, the method comprising

i) introducing said exogenous polynucleotide encoding the Lr34 adult plant pathogen resistance polypeptide into a cereal plant cell, wherein the polynucleotide is operably linked to a promoter, and

ii) generating a transgenic cereal plant from the cell, thereby producing the transgenic cereal plant, wherein the adult plant pathogen resistance polypeptide provides enhanced resistance to the plant to a plant pathogen when compared to an isogenic cereal plant lacking the exogenous polynucleotide, and wherein the pathogen is one or more of the following pathogens: *Bipolaris sorokiniana, Erysiphe graminis* f. sp. *tritici, Puccinia graminis* f. sp. *tritici, Puccinia striiformis* and *Puccinia recondita* f. sp. *tritici.*

12. The method of claim **11**, wherein the cereal plant is a wheat plant.

13. The method of claim **11**, wherein the amino acid sequence of the Lr34 adult plant pathogen resistance polypeptide is at least 98% identical to the amino acid sequence set forth as SEQ ID NO:1.

14. The method of claim **11**, wherein the amino acid sequence of the Lr34 adult plant pathogen resistance polypeptide comprises the amino acid sequence set forth as SEQ ID NO:1.

15. The method of claim **14**, wherein the cereal plant is a wheat plant.

16. A method of producing a transgenic cereal plant part which has integrated into its genome an exogenous polynucleotide encoding an Lr34 adult plant pathogen resistance polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence set forth as SEQ ID NO:1, the method comprising,

a) growing the transgenic cereal plant of claim **1**, and

b) harvesting a transgenic cereal plant part from the plant.

17. The method of claim **16**, wherein the cereal plant is a wheat plant.

18. The method of claim **16**, wherein the amino acid sequence of the Lr34 adult plant pathogen resistance polypeptide is at least 98% identical to the amino acid sequence set forth as SEQ ID NO:1.

19. The method of claim **16**, wherein the amino acid sequence of the Lr34 adult plant pathogen resistance polypeptide comprises the amino acid sequence set forth as SEQ ID NO:1.

20. The method of claim **19**, wherein the cereal plant is a wheat plant.

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